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**Studies on the effects of learning on the  
event-related potential**

**A between species comparison**

The study described in this thesis was carried out at the Nijmegen Institute for Cognition and Information (NICI), Radboud University Nijmegen, Department of Biological Psychology (Head Prof. dr. A. M. L. Coenen).

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Studies on the effects of learning on the event-related potential. A between species comparison.

Anke Sambeth

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# **Studies on the effects of learning on the event-related potential**

## **A between species comparison**

Een wetenschappelijke proeve op het gebied  
van de Sociale Wetenschappen

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## General Introduction

Previous research on learning in humans and rats has developed procedures and models about cognitive processes involved in learning. Simple paradigms such as habituation and discrimination learning were used and also rather complex paradigms such as feature discrimination learning. So far, little comparative research has been performed on the electrophysiological correlates of learning. However, by comparing the brains of humans and rats in general, and the electrical brain activity specifically, it is possible to gather new information on the functioning of the mammal brain. The aim of this thesis was to examine whether cognitive processes and their electrophysiological correlates as studied in humans are comparable to those studied in rats. The results of these studies may be used to develop, test, and improve models of cognitive functions and, in a later stage, models of cognitive dysfunctions. In this thesis, human and rat brain activity was studied using different kinds of learning paradigms.

The following sections describe habituation, discrimination learning, and feature discrimination learning, as these were the learning paradigms used in this thesis. Next, some electrophysiological components are discussed that were studied in the present thesis using both humans and rats.

### Learning paradigms

#### Habituation

In this thesis, learning will be defined as a 'change in capacity for behavior as the result of particular kinds of experience' (Lieberman, 2000). One of the most elementary forms of learning is habituation. It usually occurs if stimuli, such as tones or lights, are repeatedly presented. These stimuli initially elicit a response, called the orienting response. The first to use the term orienting response was Pavlov in 1927. He wrote: 'It is this reflex which brings about the immediate response in man and animals to the slightest changes in the world around them, so that they immediately orientate their appropriate receptor organ in accordance with the perceptible quality in the agent bringing about the change, making a full investigation of it' (as cited in Näätänen, 1979). The OR decreases and disappears rapidly if stimuli are repeatedly presented, reflecting habituation.

Habituation shows many different characteristics, some of which will be described below (see Thompson & Spencer, 1966 for review). First, habituation is



a negative exponential function of the number of stimulus presentations. A second characteristic involves the timing of the stimulus presentation. If the inter-stimulus interval is very short, habituation will develop rapidly, but it will be of relatively short life (short-term habituation). On the other hand, if the inter-stimulus interval is long, the time for habituation to occur is rather long but it will be long-lived (long-term habituation). A last feature is that habituation becomes more and more rapid in multiple sessions. At the beginning of a new session, spontaneous recovery appears, which means that the original response is at least partly recovered. During the following stimulus presentations, the response decreases faster as in the previous session. The exponential decay thus increases. This phenomenon is called enhanced re-habituation (Thompson & Spencer, 1966).

It is important to note that a response decrement does not necessarily reflect habituation. Habituation only reflects a learning process if it is not caused by factors like fatigue, a refractory period, or damage to the sensory system (Callaway, 1973; Thompson & Spencer, 1966).

Habituation can be found for many types of measures and responses to stimuli. For example, short-term and long-term habituation have been demonstrated for the human skin conductance response in response to either electric shocks (Eisenstein, Eisenstein, Bonheim, & Welch, 1990), loud tones (Siddle, Remington, Kuiack, & Haines, 1983), or vibration stimuli (Schaafsma, Packer, & Siddle, 1989). Another response that shows both short-term and long-term habituation is the acoustic startle response in humans and rats (Bradley, Lang, & Cuthbert, 1993; Haerich, 1997; Jordan & Poore, 1998; Jordan, Strasser, & McHale, 2000). A further example is heart rate, which has been shown to decrease in the case of repeated presentations of pleasant, unpleasant, and neutral scenes (Bradley et al., 1993). Finally, the brain activity habituates in response to auditory or visual stimuli in both humans and rats (e.g., Bourbon, Will, Gary, & Papanicolaou 1987; De Bruin et al., 2001; Jongsma, Van Rijn, Dirksen, & Coenen, 1998; Lammers & Badia, 1989).

Like short-term and long-term habituation, enhanced re-habituation has been found for various types of responses in both humans and animals (Johnen & Schnitzler, 1989; Kimmel, Raich, & Brennan, 1979). Waters and McDonald (1976) showed that repeated habituation sessions of the skin conductance response caused spontaneous recovery in each session, but strengthened habituation as reflected in a decreasing number of trials needed to habituate. Waters and McDonald (1975) further showed that the longer the interval between two blocks

of stimuli, the less re-habituation of the skin conductance response occurs. Kimmel and colleagues (1979) demonstrated enhanced re-habituation of the monkey's skin conductance response when changing the frequency of an auditory stimulus. The number of stimulus presentations necessary to habituate to one stimulus frequency was six, whereas this number decreased to two if a series of a stimulus with another frequency was subsequently presented. Johnen and Schnitzler (1989) found similar effects in the electrical brain activity and heart rate activity in sleeping rats. They first presented a 16 kHz stimulus, followed by a stimulus of another frequency. The response increased back to the original level at the first presentation of the stimulus with the changed frequency, but it subsequently decreased faster (Johnen & Schnitzler, 1989).

In the present thesis, the possible occurrence of short-term habituation, long-term habituation, and enhanced re-habituation will be discussed in both humans and rats in response to repeated presentations of a single stimulus.

#### *What is learned in habituation?*

According to Groves and Thompson's dual-process theory of habituation (as cited in Thompson, Groves, Teyler, & Roemer, 1973), the strength of a behavioral response is the outcome of two independent processes of habituation and sensitization. Repeated stimulus presentations either result in a decremental process, or in an incremental process, or in a combination of both. The incremental process, sensitization, occurs because stimuli are novel. The decremental process occurs as a result of familiarity with stimuli. In the process of habituation training, the incremental process, thus sensitization, grows and then decays, whereas the decremental habituation process is present throughout the training, leading to the net outcome of habituation. Thompson and Glanzman (1976) interpreted this learning phenomenon as 'learning not to respond'.

Wagner (1976) proposed a similar theory, the priming theory of habituation. Upon the presentation of a novel stimulus, an organism will first be surprised. Next, a representation of this stimulus is placed into short-term memory, in other words it is primed. Because of this priming, the next stimulus presentation will be more expected, and the organism responds less to the stimulus, reflecting habituation.

## Discrimination learning

Associative learning is the process by which we identify and learn about the causal relations within a series of events. It occurs when two events, either two stimuli or a stimulus and a response, are paired together. The pairing of two stimuli, such as the pairing of the sight of meat with an attractive taste, is called Pavlovian conditioning. The pairing of a response or behavior with a stimulus, such as the pairing of pressing a button or a remote control with the sound of music from a radio, is termed operant conditioning. In the following sections, all learning paradigms will be explained in the framework of Pavlovian conditioning. However, all these paradigms also have an operant counterpart.

In associative learning procedures, the second event is mostly biologically relevant (Lieberman, 2000). The first stimulus is initially neutral, but will be transformed into a conditioned stimulus (CS) through its being repeatedly paired with the, biologically relevant, unconditioned stimulus (US). The response to the US is not learned and is termed the unconditioned response (UR). The gradually appearing novel response to the CS, as a result of the CS-US pairings, is called the conditioned response (CR).

Sometimes it is essential or appropriate not to respond in the same way to different but similar stimuli. For instance, it is appropriate to salivate (CR) at the sight of fresh meat, but not at the sight of tainted meat. It is generally assumed that someone is able to discriminate between two stimuli if he or she responds differently to them. Discrimination learning is facilitated if the entities that have to be discriminated are easily differentiated (Lieberman, 2000); e.g., 1000 Hz and 8000 Hz auditory stimuli are easier to differentiate than 1000 Hz and 2000 Hz stimuli.

In this thesis, two experiments are discussed in which humans and rats had to learn to discriminate between two stimuli.

### *What is learned in associative learning?*

Two types of models have been proposed regarding the processes underlying discrimination learning, and associative learning in general, namely the Rescorla-Wagner model (1972) and a configural model (Pearce, 1987, 1994, 2002). Both models assume that the key to learning is surprise. The more surprising the US, the more will be learned about the predictive value of the CS. The amount of conditioning on every CS-US pairing depends, therefore, on the subject's expectation that the US will occur together with the CS. The increase will become

progressively smaller upon repeated stimulus presentations, resulting in a negative learning curve (Rescorla & Wagner, 1972).

A difference between the two models is that, in the model of Rescorla and Wagner (1972), stimulation will lead to the activation of a set of elements from memory, namely the CS and US elements. Subjects learn that the CS predicts the US. In contrast to this model, the configural model of Pearce (1987, 1994, 2002) assumes that a compound of several stimuli, such as the odor and the context in which it is presented, are activated as a unitary stimulus. What subjects learn is that the presentation of this unit or configuration predicts the US.

### **Feature discrimination learning**

In the examples of discrimination learning mentioned above, direct associations are established between the CS and the US. Under some conditions, however, a stimulus may be indirectly related to another stimulus or to a group of stimuli. For instance, in a Pavlovian feature-positive discrimination procedure, a target stimulus (the CS) is followed by the US if it is preceded by a feature stimulus, and it is not followed by the US if presented alone. In this case, an association is established between the target stimulus and the US, and this association is in turn modulated by the feature stimulus. The feature, thus, is not directly related to any one of the stimuli, but to the target-US association. The feature is said to ‘set the occasion’ for the presence of the target-US link (for reviews see Holland, 1992; Schmajuk & Holland, 1998). It is, however, possible that the feature also establishes a direct association with the US, together with the modulatory association. This direct association may exist independently from the modulatory association (Holland, 1992).

Control manipulations like counter-conditioning or extinction are commonly implemented on the feature stimulus (for a review see Holland, 1992; Schmajuk & Holland, 1998) in order to determine whether the feature indeed possesses modulatory properties, or rather only direct associations. In case of both a counter-conditioning and an extinction procedure, the associative value that was potentially established with respect to the feature stimulus in the first part of the procedure is explicitly changed in a second part (Holland, 1991). For example, in counter-conditioning, a subject first learns that the presentation of a feature (e.g., red light) before the target (e.g., tone) is followed by the US (food), and presentation of another feature (e.g., green light) together with the same tone is not followed by food. Subsequently, the red light is presented alone and no longer

followed by food, whereas single presentations of the green light are followed by food. In a final test phase, the red and green lights are again presented together with the tones. What will a subject do? If the subject responds to the tone after the red light, the feature had functioned as an occasion setter in the first phase of the experiment. If, however, the subject only responds to the tone after the green light, responding is based on direct associations between the lights and (no) food.

A further strategy is the transfer test. In this test, the potential of the feature to modulate responding to other targets is examined. Transfer should be selective to targets that have been trained in similar tasks if the feature has modulatory properties. Instead, if the feature is directly associated with the US, its controlling property would transfer to any target. Several researchers indeed showed that transfer of behavioral control is stronger for targets that already have been trained in another occasion-setting discrimination than for other types of targets (Bonardi, 1998; Bonardi & Hall, 1994; Holland, 1989, 1991, 1995b).

Feature discrimination procedures have extensively been used in animals (for reviews see Holland, 1992; Schmajuk & Holland, 1998). A number of experiments have also been performed in humans using these kinds of tasks (Baeyens, Crombez, De Houwer, & Eelen, 1996; Baeyens, Hendrickx, Crombez, & Hermans, 1998; Baeyens, Vansteenwegen, Hermans, Vervliet, & Eelen, 2001; Dibbets, Maes, & Vossen, 2002; Dibbets, Maes, Van den Berg, De Wit, & Vossen, 2002; Hardwick & Lipp, 2000; Young, Johnson, & Wasserman, 2000). Although the first two studies of Baeyens and colleagues (1996, 1998) did not reveal evidence for modulatory associations of the feature stimulus, the other studies (Baeyens et al., 2001; Dibbets, Maes, & Vossen, 2002; Dibbets, Maes, Van den Berg, De Wit, & Vossen, 2002; Hardwick & Lipp, 2000; Young et al., 2000) did find at least partial support for occasion setting in feature discrimination tasks using extinction and counter-conditioning control procedures.

As fewer studies have employed human subjects in feature discrimination tasks, it was first assessed whether the feature indeed possesses occasion-setting properties in humans. In a next experiment, it was examined whether the electrophysiological components of humans and rats are comparable in a feature-positive discrimination task.

### *What is learned in feature discrimination tasks?*

One of the assumptions of occasion-setting models (e.g., Holland, 1992; Nelson & Bouton, 1997) is that associations are established between the target and the US. These associations can be either excitatory, inhibitory, or a combination of

both. The feature in turn modulates these target-US associations. For example, in the model described by Holland (1992), the feature X indicates that the target A is reinforced. It positively activates the excitatory A-US association. In the model of Nelson and Bouton (1997; Bouton & Nelson, 1998), on the other hand, the feature affects an inhibitory target-US link, namely by shutting it down. This also leads to excitation.

Another explanation of behavior in feature discrimination tasks comes from Pearce (1987, 1994; Pearce & Bouton, 2001). As outlined in the discrimination learning section, the feature and target can be seen as one configural element. Responding depends on the strength of the association between the compound and the US, together with any associative strength that generalizes to it from similar compounds that have been conditioned. The effect of the various control procedures on responding to the compound used during conditioning may be argued to be low because of a limited generalization between the original and newly trained compound (Pearce, 1987).

## **Electrophysiology**

### **Electroencephalography**

Jasper (1958) introduced the 10-20 system of electrode placement, which is now commonly used for electrode positioning in human electrophysiological research. In this system, electrodes are arranged according to standard landmarks on the skull and the leads are proportional to skull size and shape. In this way, the electrical brain activity (EEG) can be measured from distinct cortical parts of the brain. Although the brain of monkeys is not entirely comparable to that of humans, research in monkeys often describes electrode sites identical to those used in human research (Arthur & Starr, 1984; Glover, Onofri, Ghilardi, & Bodis-Wollner, 1986; Pineda, Westerfield, Kronenberg, & Kubrin, 1997). Unfortunately, the brain of rats differs too much from that of humans to use the same positioning system. Therefore, in rats the anatomical coordinates related to bregma are used to specify the electrode locations (e.g., Coenen, 1995; De Bruin et al., 2001; Paxinos & Watson, 1998).

## **Event-related potential**

The event-related potential (ERP) characterizes changes in the EEG signal in response to a definable event such as a sensory stimulus. This change in signal is small compared to the amplitude of the background EEG. Therefore, EEG samples of several stimulus presentations are averaged, time-locked to the start of a stimulus. It is assumed that EEG activity that is not time-locked to the event will tend to average to zero, and the residual waveform after averaging then represents activity related to the stimulus (Coenen, 1995; Dawson, 1954).

The ERP of a subject has been supposed to be stable during experiments, although differences between individual subjects can be observed. Therefore, averaging procedures have been useful tools to detect the waveforms. A disadvantage of the averaging process might, however, be that changes in the ERP-response over time remain undetected in unstable conditions. Therefore, new methods have been developed to extract the direct response to a stimulus from the background EEG on a trial-by-trial basis. One of these methods is wavelet denoising (e.g., Quian Quiroga, 2000).

In this project, both signal averaging and single-trial analyses were used in order to 1) learn about the comparability of humans and rats under relatively stable conditions after learning is completed and 2) investigate whether the ERP changes similarly in the course of an experiment in both humans and rats.

## **(Neuro)physiological vs. Functional approach**

Several components can be distinguished in the ERP waveform. The most straightforward manner to distinguish the components is to concentrate on a certain feature of the waveform, such as a peak or a trough, and to determine its polarity, latency, and amplitude. In addition to this method, physiological or functional approaches to component definition are usually implemented (Coles & Rugg, 1995; Gaillard, 1988).

In the (neuro)physiological approach, the distribution on the scalp is a critical factor in deciding whether or not two ERP waves reflect the same component (Coles & Rugg, 1995; Gaillard, 1988). Two waves that have the same scalp distribution are assumed to be generated by one and the same neurophysiological structure. If the scalp distribution of two components is different, it is suggested that they have different neural generators.

In the functional or information-processing approach, the components are defined in terms of the psychological factors that determine their occurrence (Coles & Rugg, 1995; Gaillard, 1988). The amplitudes and latencies are studied as a function of the task variables that relate to certain cognitive processes.

In this thesis, a simple approach in defining components is used, because for at least some components, neither the location of the neural generator, nor the functional meaning are entirely clear. Some information on the neural generators of some components is available, but this information is not yet conclusive, especially for the ERP components found in rats. The components will be indicated by their polarity and order of occurrence in both humans and rats, for example, the P3 is the third positive component.

## **ERP components in humans**

### *Readiness Potential*

The first component that is discussed is a component that does not follow the presentation of a stimulus: it precedes a voluntary movement. The Readiness Potential (RP) is a negative wave that increases until just before the voluntary movement is executed. Unlike the other components that are discussed, this component is labeled according to its psychological meaning, namely 'being ready to perform'.

The RP has been associated with motor preparation (Coles & Rugg, 1995). Brunia (1993), however, suggested that it is not mere motor preparation. As the execution of the movement is the objective of a preparatory process, motor preparation, and thus the RP, can be seen as attention selectively directed to the output system (Brunia, 1993).

Motor preparation usually occurs in self-paced movement tasks. It may, however, also be found in tasks with fixed interstimulus intervals. Starr, Sandroni, and Michalewski (1995) found a RP in a simple fixed interval discrimination task in which participants had to respond to one, but not to another stimulus. The RP was only found if participants had to press a button in response to a stimulus and not if the participants had to count the stimuli. This indeed suggests a motor component in this potential.

### *P50/P1*

The human P50 (further called P1) is a positive wave at around 50 ms after stimulus onset. It is usually studied in experiments examining a so-called sensory



gating paradigm, in which two identical stimuli are presented with an interval of 500 ms. The amplitude of the P1 to the second stimulus is usually smaller than to the first (e.g., Boutros & Belger, 1999; Grunwald et al., 2003) in healthy subjects, although this amplitude relation is disturbed in patients suffering from schizophrenia (e.g., Adler, Hoffer, Griffith, Waldo, & Freedman, 1992; Freedman, Adler, Waldo, Pachtman, & Franks, 1983; Zouridakis, Boutros, & Jansen, 1997).

A number of studies have addressed the issue of the meaning of the P1 in terms of information processing, such as attention, using choice-reaction-time tasks (Jerger, Biggins, & Fein, 1992; White & Yee, 1997). In the task of Jerger and colleagues (1992), single or paired clicks were presented and the participants received the instruction to attend either the first, or the second click. The presence or absence of the second click provided the important information and the researchers hypothesized that, if attention modulates the P1, the amplitude in response to the second stimulus should be as large or even larger than the amplitude to the first. However, they showed that the amplitude of the P1 was decreased at the second compared to the first stimulus in the pairs, indicating that the P1 was not affected by attention modulation (Jerger et al., 1992). White and Yee (1997) showed the same effect in a similar choice-reaction-time task. Kho and colleagues (2003) used either pairs of two clicks or pairs of a click and a tone, and participants were instructed to pay attention to the tones in one condition and were distracted in another. Kho and co-workers demonstrated that the amplitude reduction in response to the second stimulus compared to the first was identical for both conditions. Therefore, the amplitude of the P1 gating was again not modulated by attention. However, the P1 in response to the first stimulus differed between the attention and distraction condition, indicating that the P1 may be modulated by attention after all. These studies have contributed to the general view that P1 gating is a pre-attentive process, not sensitive to cognitive operations (e.g., Coles & Rugg, 1995; Kho et al., 2003), whereas the P1 to the first stimulus in a pair may be sensitive to attention (Kho et al., 2003).

### *N100/N1*

The N100 (further labeled N1) is a large negative component with a mean latency of around 100 ms (70-140 ms) that shows its maximum amplitude at frontal and central electrode positions. This component can be elicited in many kinds of tasks, but is often studied in experiments intended to examine selective or divided attention processes.

The N1 is usually more negative in response to attended than to unattended stimuli (e.g., García-Larrea, Lukaszewicz, & Mauguière, 1992; Golob, Pratt, & Starr, 2002; Jerger et al., 1992; Kho et al., 2003; Näätänen, 1990; Rockstroh et al., 1996; White & Yee, 1997). If a participant has to divide the attention over several stimuli at the same time, the amplitude of the N1 is intermediate between the attended and unattended conditions (Kok, 1997).

If attention needs to be paid to several kinds of stimuli in an active task at different times, the amplitude of the N1 does not differ between the different attended trial types (Kok, 1997; Ragot & Fiori, 1994). Task load, however, has a general effect on the N1 component, decreasing the amplitude with increasing load (Kramer, Trejo, & Humphrey, 1995; Ullsperger, Freude, & Erdmann, 2001). As the N1 is equally large at all stimuli that are attended (Kok, 1997), and as it is affected by task load, it is suggested that the N1 primarily reflects perceptual capacity, the capacity available to perceive stimuli. This capacity is said to be invested in the selection of the physical properties of the attended stimulus (Kok, 1997; Näätänen & Picton, 1987).

### *P200/P2*

The P200 (further labeled, P2), which occurs at around 200 ms after stimulus onset, is one of the least studied components. Sometimes the N1 and P2 are seen as one complex, the N1-P2 complex (e.g., Picton, Hillyard, Krausz, & Galambos, 1974). This might indicate that the N1 and P2 share some characteristics, for example, that they are both modulated by attention.

In a cued attention task used by Golob and colleagues (2002), participants first received a cue about the location of a test stimulus, and subsequently the test stimulus. Cues that predicted the location of the test stimulus correctly elicited smaller P2 component amplitudes than did neutral cues not predicting the location or invalid cues (Golob et al., 2002). Golob and colleagues suggested that the P2 was increased as a result of attentional cuing in the latter two cases, because participants needed to focus their attention longer in order to receive information about the test stimulus. Thus, the P2 component may indeed reflect attention processes.

The P2 component is, however, not only modulated by attention. It has been shown that the amplitude of the P2 component was more positive in response to infrequent than frequent stimuli, whether or not a participant had to respond to it (Lang, Kotchoubey, Lutz, & Birbaumer, 1997), thus whether or not attention

had to be paid to the stimulus. More research is needed to unravel the functional significance of this component.

### *N200/N2*

The second negative component, the N200 (further named N2), occurs between 200 and 300 ms after the onset of a stimulus. Like the N1 component, the N2 component reaches its maximum at frontal to central areas.

According to Fitzgerald and Picton (1983), who studied the N2 in tasks of differing complexity, this component reflects controlled deviance detection. In their experiment, the participants had to detect deviant stimuli in a train of frequent stimuli. Fitzgerald and Picton showed that the amplitude of the N2 was increased to the deviant stimuli. They further showed that the N2 component increased as deviance detection became more difficult and that it was only large in easy tasks if a stimulus was made highly improbable.

Another explanation of the N2 component comes from Kasai and colleagues (2002), who used a task similar to that used by Fitzgerald and Picton (1983). The participants had to attend to relevant stimuli presented at one ear only and not to relevant stimuli at the other ear or irrelevant stimuli. Kasai and co-workers (2002) found that the N2 component was even present in the unattended condition and it was, therefore, concluded that the N2 reflects an attention switching process.

The last few years, the N2 component has been studied frequently in so-called Go/NoGo tasks. The general element in these tasks is that participants have to respond to a Go stimulus, but to withhold responding to the NoGo stimuli. The Go and NoGo stimuli can either be test stimuli in cued tasks, or stimuli in simple discrimination tasks. The N2 has been shown to be more negative if participants have to refrain from responding than if they have to make a response. Therefore, it is suggested that the N2 component reflects response inhibition processes (Bokura, Yamaguchi, & Kobayashi, 2001; Bruin & Wijers, 2002; Falkenstein, Hoormann, & Hohnsbein, 1999; Fox, Michie, Wynne, & Maybery, 2000; Kopp, Mattler, Goertz, & Rist, 1996; Naito & Matsumura, 1996).

However, whether the N2 indeed reflects response inhibition is not entirely clear thus far (Bruin, Wijers, & Van Staveren, 2001; Falkenstein, Koshlykova, Kiroj, Hoormann, & Hohnsbein, 1995). For instance, Falkenstein and colleagues (1995) demonstrated that the N2 effect was only present when using visual stimuli, but not when using auditory stimuli, although response inhibition should be modality independent. Bruin and co-workers (2001) showed that priming information did not influence the amplitude of the N2 at NoGo stimuli in a

Go/NoGo task. The amplitudes were equally negative at NoGo stimuli with or without previous information about whether the participants had to respond, suggesting that response inhibition cannot account for the generally found N2 effects. Bruin and colleagues speculated that it may rather be response activation that affects the N2 amplitude, although they did not have any arguments for this statement. More research is needed to clarify this controversy.

### *P300/P3*

The P300 (further labeled P3) component is a relatively large, positive component (10-20  $\mu$ V) with a maximum amplitude at parietal electrode positions and with a mean latency of around 300 ms (250-500 ms). This component is often elicited within simple discrimination tasks. However, extremely alerting or novel stimuli may also produce this P3, although with a somewhat earlier latency. Therefore, two kinds of P3 have been distinguished. The early P3 in response to novel or alerting stimuli is called P3a, whereas the P3 in response to discrimination tasks is usually labeled P3b (e.g., Coles & Rugg, 1995; Picton, 1992; Polich & Kok, 1995).

### P3b

The first to report the P3 were Sutton, Braren, Zubin, and John (1965). Presenting both auditory and visual stimuli, and further varying the amount of information about which stimulus was going to be presented, Sutton and colleagues showed that the amplitude of the P3 was more positive if no information was provided before a stimulus than if information was presented. They further found that the amplitude of the P3 component was influenced by several factors, such as information content, stimulus probability, and sequence. Several years after the first demonstration of the P3 by Sutton and colleagues, the P3 was 're-labeled' P3b.

Nowadays, the P3b is often studied in oddball tasks. In these tasks, frequently occurring standard stimuli are interspersed with infrequently occurring target stimuli and participants are asked to count or press a button in response to the targets. Ritter and Vaughan (1969) were the first to use this task and showed that the amplitude of the P3b was more positive in response to targets than to standard stimuli. Furthermore, Vaughan and Ritter (1970) showed that the amplitude was most positive at centro-parietal electrode sites in this task.

Oddball studies have shown that the P3b component is more positive in response to stimuli with a low probability than to stimuli with a high probability

of occurrence in a task (e.g., Katayama & Polich, 1996; Polich, 1990a, 1990b; Polich, Ellerson, & Cohen, 1996). Not only this so-called global probability, but also the local probability (sequence) and, furthermore, inter-stimulus interval have an effect. The more standard stimuli are presented between two following targets and the longer the interval between stimuli, the larger the P3b will be in response to the targets (Gonsalvez et al., 1999; Strüber & Polich, 2002).

The P3b has also been studied in other kinds of tasks, such as the continuous performance test. In this task, participants have to respond to a letter (say X) when it is preceded by another letter (say A) and to withhold responding when another letter follows the A. It has been shown that the amplitude of the P3 component to X is larger than that to other letters presented after A, at parietal electrode sites (Bokura et al., 2001; Tekok-Kilic, Shucard, & Shucard, 2001).

Other means to examine the P3b component are dual-task studies. For instance, if participants have to perform a task, such as detecting different kinds of targets and acting correspondingly to each of the targets, and if an oddball task is presented at the same time, the amplitude of the P3 in response to the oddball stimuli is decreased compared to the amplitude of the P3 in an oddball-alone situation (Kramer et al., 1995). Other dual task studies have confirmed the amplitude decrements of the P3 (e.g., Singhal, Doerfling, & Fowler, 2002; Ullsperger et al., 2001).

### P3a

The distinction between P3a and P3b component was first made in 1975 (Courchesne, Hillyard, & Galambos, 1975; Squires, Squires, & Hillyard, 1975). Squires and colleagues (1975) studied the effects of unpredictable intensity or frequency shifts of auditory stimuli and found that an early frontally maximal P3, the P3a, was present at 220-280 ms after stimulus onset in these trials, whether the stimuli were attended to or not. This component was different from the P3b, as the latter was only strongly present at attended infrequent and unpredictable shifts.

Courchesne and co-workers (1975) studied the P3 in a visual three-stimulus oddball task. The participants had to respond to infrequent targets, but not to the frequent standards. Additionally, Courchesne and colleagues interspersed the targets and standards with infrequent novel stimuli, and found a P3a component to these novel stimuli, which they called novels-P3. As in the study of Squires and colleagues (1975), the novels-P3 component was largest at frontal electrode sites.

More recently, it has been suggested that the P3a reported by Squires and colleagues and the novelty-P3 described by Courchesne and co-workers are one and the same component (Simons, Graham, Miles, & Chen, 2001), and that the P3a is indeed elicited in response to novel stimuli (e.g., Barcélo, Perianez, & Knight, 2002; Friedman, Cycowicz, & Gaeta, 2001). However, the P3a can also be found to infrequent unattended stimuli in three-stimulus oddball tasks (Goldstein, Spencer, & Donchin, 2002; Katayama & Polich, 1998), as they probably alert the participant unintentionally in the same way as novel stimuli do.

### Theoretical considerations

There has been much debate about the functional significance of the P3, especially the P3b, component. Some of the theories on the meaning of the P3b component will, therefore, be discussed in the next section.

Shortly after the ‘discovery’ of the P3b, a few researchers started to investigate the conditions that affect the amplitude of this component. It was found that its amplitude increased with decreasing global stimulus probabilities (Duncan-Johnson & Donchin, 1977; Squires, Wickens, Squires, & Donchin, 1976). For instance, if the target stimulus in an oddball task was only presented in 10% of the trials, the amplitude was more positive than if the probability was 30%. Furthermore, these studies showed that the preceding sequence of stimuli is of importance (local probability). The more standards were presented before the target, the more the amplitude of the P3b increased at both targets and standards just preceding the target (Duncan-Johnson & Donchin, 1977; Squires et al., 1976).

On the basis of their results, Squires and colleagues (1976) formulated an ‘expectancy model’ and assumed expectancy to be controlled in a linear fashion by three aspects: 1) the memory for event frequency within the preceding stimulus sequence 2) the specific structure of the preceding sequence, and 3) the global probability of the event.

Another view comes from Donchin (1981). He suggested that the P3b reflects the manifestation of the process whereby mental schemas are revised. According to Donchin, a mental schema is a large and complex map representing all available data of the environment. This schema is the reservoir that is necessary to perform whatever tasks require active processing at any time. If a stimulus is presented, it is evaluated and if the information is new, the schema will be revised. It is this revision that reveals a large P3b (Donchin, 1981). In case of ‘old’ information, such as the frequently presented standard in the oddball task, no revision will be needed and, therefore, the P3b will be small or even absent.

Johnson (1986) proposed the ‘triarchic model’ of P300. According to this model, one of the three dimensions influencing the P3b amplitude is information transmission. If the information that is provided to a participant is not properly used because of inattention or ambiguity, the P3b amplitude cannot be large or may even be absent.

Information transmission itself has an important influence on the other two dimensions, subjective probability and stimulus meaning. Without transmission, the latter two dimensions cannot come into effect. Subjective probability and stimulus meaning independently and additively affect the P3b amplitude. This means that their amplitude contributions either simultaneously increase or decrease the amplitude in equal proportions (Johnson, 1986).

To a large extent, Johnson’s model (1986) resembles the expectancy model of Squires and colleagues (1976), as probability plays an important role in both models. The model of Johnson additionally assumes that the meaning and importance of a stimulus will affect the amplitude of the P3b. The model, however, differs from that of Donchin (1981) in the interpretation of memory. According to Donchin (1981), all available data of the environment are kept in memory (the schema), whereas only probability and sequence factors are in memory in the view of Johnson (1986).

A last model that will be described is the model presented by Kok (2002). This model (see Figure 1) assumes several major determinants of P3b amplitude, namely stimulus probability, task relevance and task difficulty. Furthermore, two underlying mechanisms, that is, attention and working memory, are affected by these three determinants. For instance, low probability or instructions about task relevance will automatically draw the attention to stimuli, affecting the P3b amplitude by increasing it. Task difficulty, however, may counteract this process (Kok, 2002). The attention and working memory systems must come into contact in order to affect the last mechanism, the event categorization process. This is the process that involves a comparison of the external stimulus to internal representations and in the end determines the P3b amplitude.

Kok’s model includes all of the aspects of the other models. Probability plays an important role, as well as its relevance (Johnson’s stimulus meaning). Furthermore, like Donchin (1981) suggested, a mental schema is updated (e.g., event categorization). One needs to search for the significance of the attended stimulus in working memory in order to properly categorize the stimulus. However, it is still not entirely clear which of the factors affects the P3b component most.

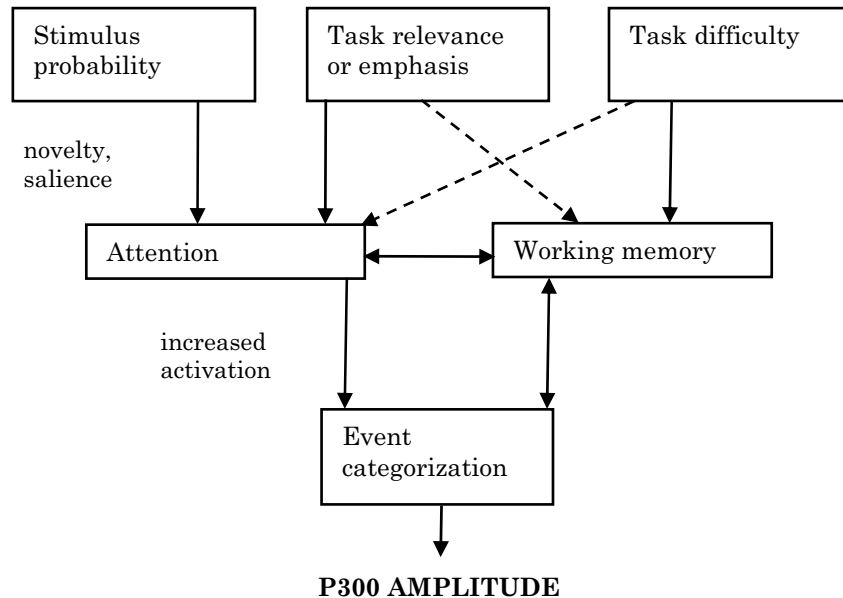


Figure 1. Event categorization model of Kok (2002).

### ERP components in rats

The following cortical auditory ERP components can be distinguished in rats at approximately the following times after stimulus onset: a P20 (P1), N60 (N1), P120 (P2), N160 (N2), and P300 (P3) (e.g., Ehlers & Chaplin, 1992; Ehlers, Kaneko, Robledo, & Lopez, 1994; Ehlers, Wall, & Chaplin, 1991; Hurlbut, Lubar, & Satterfield, 1987; Meeren, Van Cappellen van Walsum, Van Luijtelaar, & Coenen, 2001; Shinba, 1997; Slawewski, Walpole, Somes, Li, & Ehlers, 1999): three positive and two negative waves. The order of polarity of the components is equal to those found in humans. An important difference between the human and rat ERP, however, is that the first four components found in rats occur much earlier than those observed in humans. Only the P3 component has an equal latency, although several studies using rats have shown a P3 component at latencies between 220 and 240 ms (Galicía et al., 2000; Yamaguchi, Globus, & Knight, 1993).

The functional significance of these components is not clear yet. Several studies that used an oddball task in rats showed that the P3 component was more



positive in response to the target stimuli than to the standards (Brankač, Seidenbecher, & Müller-Gärtner, 1996; Hurlbut et al., 1987; Jodo, Takeuchi, & Kayama, 1995; Shinba, 1997, 1999; Shinba, Andow, Shinozaki, Ozawa, & Yamamoto, 1996; Takeuchi et al., 2000; Yamaguchi et al., 1993). As the target P3 was larger than the standard P3, and as the latency of the P3 was equal to the human P3b latency in these tasks, several researchers (Jodo et al., 1995; Shinba, 1997; Takeuchi et al., 2000) concluded that the rat P3 corresponds to the human P3, or suggested that a common neural circuitry may be involved in the generation of the human and rat P3 (Yamaguchi et al., 1993). However, as the rat P3 has only been studied during oddball tasks up to now, it is too early to draw any definite conclusions about the functional significance of this component.

Another component that showed significant differences between targets and standards in at least one of the oddball studies is the N1 component (Takeuchi et al., 2000). The amplitude was more negative in response to the targets than to the standards. Takeuchi and colleagues (2000) suggested that this N1 task effect reflected some aspect of information processing, although it was not clear yet whether this corresponded to that reflected in the human N1.

In this thesis, similar tasks were used in both humans and rats in order to learn more about the functional significance of the ERP components frequently found in rats.

## **Learning paradigms in combination with electrophysiology**

### **Habituation and ERPs**

Both short-term and long-term habituation have been studied in human ERP research. In passive paradigms, the N1, P2, and P3a components demonstrate short-term habituation in response to stimuli in easy or passive conditions (e.g., Bourbon et al., 1987; Lutzenberger, Schandry, & Birbaumer, 1979; Maclean, Öhman, & Lader, 1975; Öhman, Maclean, & Lader 1975; Polich & McIsaac, 1994). Long-term habituation has been found for both the N2 and P3a components in passive tasks (Pan, Takeshita, & Morimoto, 2000; Polich, 1989).

Several ERP studies examined short-term habituation in rats and showed that the N1 and P2 component decreased with repeated stimulation (De Bruin et al., 2001; Quian Quiroga & Van Luijtelaa, 2002; Shucard & Specht, 1996; Specht & Shucard, 1996). No studies on long-term habituation have been performed in rats so far.

## **Discrimination tasks and ERPs**

Numerous ERP studies have been performed in humans using the oddball task (e.g., Gonsalvez et al., 1999; Katayama & Polich, 1996; Polich, 1990a, 1990b; Polich et al., 1996; Ritter & Vaughan, 1969; Strüber & Polich, 2002). In this task, the P3b component is larger in response to the target than to the standard stimulus. Not only the P3b is increased in response to the targets, but also the amplitudes of the N1 (e.g., Garcia-Larrea et al., 1992), P2 (Lang et al., 1997), and N2 components (e.g., Fitzgerald & Picton, 1983) are more pronounced during targets than during standards.

Oddball studies have also been performed in rats, in which similar results are found as in humans with regard to the P3 component. Its amplitude is more positive in response to the target than to the standard stimuli (Brankačk et al., 1996; Hurlbut et al., 1987; Jodo et al., 1995; Shinba, 1997, 1999; Shinba et al., 1996; Takeuchi et al., 2000; Yamaguchi et al., 1993).

## **Feature discrimination learning and ERPs**

No previous human studies explored the ERP in response to feature-positive discrimination tasks. However, tasks relatively similar to the occasion-setting procedure have been used in humans. Using the continuous performance test, in which a cue (the feature) gives information about how to respond to the test stimulus (the target) that will occur, the P3b component is more positive in response to the targets that require a response than to targets to which no response has to be executed (Bokura et al., 2001; Tekok-Kilic et al., 2001).

Studies using the Go/NoGo task, which is actually the same as many of the continuous performance tests, have shown that the amplitude of the N2 component is, unlike the P3 component, decreased during the targets that require a response. In other words, if participants do not have to respond to a target, the amplitude is more negative, which suggests response inhibition (Bokura et al., 2001; Bruin & Wijers, 2002; Falkenstein et al., 1999; Fox et al., 2000; Kopp et al., 1996; Naito & Matsumura, 1996).

So far, no studies examined ERP components in rats subjected to feature discrimination tasks.

## **Aim and outline of the present studies**

The aim of the present thesis was to directly compare the electrophysiology of humans and rats using different learning paradigms, because such research may be a good method to unravel the physiological substrates of cognitive processing. The purpose of the experiments described in this thesis was to compare the human and rat auditory ERP components using different learning procedures. The first chapters (Chapters 2 and 3) describe simple learning as reflected by habituation. A second series of experiments (Chapters 4, 5, and 6) was performed to examine the correspondence of the human and rat ERPs under simple discrimination learning. Finally, a last series of experiments (Chapters 7 and 8) describes relatively complex learning by using feature discrimination learning procedures. This thesis further intended to relate the human and rat ERP components to cognitive processes as implied in the learning procedures, which is described in the general discussion.

The aim of the first experiment was to explore human and rat ERP components in the most elementary learning paradigm, habituation. By presenting several blocks of auditory stimuli, it was examined whether the amplitudes of ERP components decreased within a block (short-term habituation) and between blocks (long-term habituation). Results of this experiment are described in Chapter 2. Furthermore, one of the human ERP components demonstrated enhanced re-habituation and these results are described in Chapter 3.

Next, the ERP components that are elicited in both humans and rats during simple discrimination procedures were specified. Furthermore, it was examined whether task effects on the ERP were similar in the two species. In one experiment, described in Chapter 4, the subjects had to perform an oddball task. In the experiment described in Chapter 5, it was assessed whether learning affects the human ERP in an oddball task in a similar way as it does in the animal counterpart described in Chapter 4. Finally, in Chapter 6, the extent to which the interval between stimuli in oddball and single stimulus tasks affects the amplitude of the human ERP components was studied. The participants were assigned to three conditions. The participants in the oddball condition had to respond to the targets, but not to the standards. Those in the two single-stimulus conditions (short vs. long interstimulus interval) responded to every stimulus.

A last series of experiments was performed to assess which ERP components are present in the humans and rats during complex forms of learning. As it is not

yet entirely clear whether occasion setting occurs in humans, two serial feature discrimination tasks were first performed using only human participants. Results of these two experiments are described in Chapter 7. The next experiment compared the ERPs of humans and rats in a serial feature-positive discrimination task and the results of this experiment are described in Chapter 8.

In Chapter 9, the findings of the present studies are discussed and summarized. The correspondence of several of the ERP components observed in the two species was evaluated. Finally, it was attempted to relate some of the ERP components to particular cognitive processes.



## **Chapter 2**

# **Effects of stimulus repetitions on the event-related potential of humans and rats**

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### **Abstract**

The present study compared the effects of repeated stimulus presentations on the event-related potential (ERP) of humans and rats. Both species were presented with a total of 100 auditory stimuli, divided into four blocks of 25 stimuli. By means of wavelet denoising, single-trial ERPs were established in both humans and rats. The auditory ERPs were characterized by the presence of two positive and two negative waves in both humans and rats, albeit with different latencies in the two species (P1, N1, P2, and N2). The results showed decreased amplitudes within blocks for the N1, P2, and N2 components in humans and for the N1 and P2 components in rats. Decreased amplitudes across blocks were found for the N2 component in humans and for the P2 and N2 components in rats. In both humans and rats, response decrements within a block were thus most prominent for the early ERP components, whereas the changes across blocks were most prominent for the later components. These results suggest a correspondence of the ERP correlates of elemental stimulus processing between humans and rats. It is further suggested that the observed amplitude reductions may reflect habituation and / or recovery cycle processes.

Descriptors: event-related potential, habituation, human, rat, recovery cycle, single trial, stimulus repetition, wavelet analysis

Event-related potentials (ERP) are averaged electroencephalographical (EEG) potentials triggered by, and time-locked to, sensory stimuli. In the human ERP, the P50 (P1), N100 (N1), P200 (P2), N200 (N2), and two P300 (P3) components can be distinguished. Previous rat studies have shown P20, N60, P120, and N160 (P1, N1, P2, and N2, respectively) components in rats in response to the presentation of auditory stimuli (e.g., Meeren, Van Cappellen van Walsum, Van Luijtelaar, & Coenen, 2001; Sambeth et al., 2003). A P3-like component may also be found in active tasks in rats (e.g., Ehlers, Kaneko, Robledo, & Lopez, 1994; Jodo, Takeuchi, & Kayama, 1995; Shinba, 1997, 1999).

In humans, the amplitudes of several ERP components have been shown to decrease with repeated stimulus presentation. For instance, the amplitude of the human N1 and P2 components has been shown to decrease in response to stimuli in both simple active or passive paradigms (e.g. Maclean, Öhman, & Lader, 1975; Öhman, Maclean, & Lader, 1975; Lutzenberger, Schandry, & Birbaumer, 1979; Bourbon, Will, Gary, & Papanicolaou, 1987). The human N2 was found to decrease within a simple active task (Polich, 1989), and the P3 amplitude decreased in a passive listening and oddball task (Bourbon et al., 1987; Polich & McIsaac, 1994) and in simple active oddball tasks (Polich, 1989; Pan, Takeshita, & Morimoto, 2000).

Several ERP studies have examined the effects of stimulus repetitions on ERP components of rats. Shucard and Specht (1996) showed that peak-to-peak amplitudes of the N40/P90 component decreased from the first to the second tone in a passive condition, which was not due to increased latency variability (Specht & Shucard, 1996). The studies of De Bruin et al. (2001) and Quiñ Quiroga and Van Luijtelaar (2002) also showed amplitude decrements at a N50 component. Furthermore, Jongsma and colleagues (1998) found amplitude decrements at a positive component of a similar latency, a P50 component, and at N23, P30, and P150 components.

So far, only one study has explicitly attempted to directly compare ERP components of humans and rats in a passive procedure (Rockstroh et al., 1987). Using a passive listening task, Rockstroh and colleagues (1987) showed that peak-to-peak amplitudes of the human N150/P340 (the common N1/P3) and the rat N80/P130 (the N1/P2) components decreased with increasing number of stimulus presentations. Whereas the P3 component was found in the humans, no such component was present in the rats. However, a limitation was that recordings in the animals were made during sleep and those of the human participants during wakefulness.

In the present study, we compared the ERP components of humans and rats in a passive task during wakefulness to further study whether the ERPs of humans and rats change in a similar way in the course of an experiment. Both human and rat subjects were presented with a total of 100 auditory stimuli, divided over four blocks of 25 stimuli. We examined changes in ERP responses both within and across blocks.

## **Materials and Methods**

### **Subjects**

#### *Humans*

Forty-eight healthy students (12 men and 36 women, mean age 22 years) from the University of Nijmegen, The Netherlands, participated in the experiment. They received course credits. The participants were only allowed to take part in the study if they were healthy, did not use medication, and had no psychiatric history. Participants who agreed to participate signed an informed consent.

#### *Rats*

Thirty-two nine-month old male Wistar rats served as subjects. They were maintained on a 12-12 h light-dark cycle with lights off at 8.00 a.m. The animals were singly housed in Plexiglas cages in which they had unlimited access to food and water. Animals were handled daily from one week before until the day of the experiment. The study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethical committee for animal experiments was obtained.

### **Apparatus**

The human participants were tested in a sound-attenuating cubicle (inside dimensions: 2 x 2.2 x 2 m). A speaker used for presenting auditory stimuli was located to the right side of the participant. The auditory stimulus consisted of a 1500-Hz, 70-dB pure tone that had a 10 ms rise and fall time. Each tone presentation lasted 1 s. The present experiment was part of a larger study performed at the University of Nijmegen. For reasons not related to the present experiment, half the participants were seated in the light and the other half in the dark. As the statistical analysis showed no main effect of illumination, the pooled data of these participants are reported in this paper.



The animal subjects were tested during the dark period in a set of eight identical operant boxes in which EEG recordings could be made. Each box measured 25 x 24 x 40 cm. The front and back walls were clear Plexiglas; the right side wall and floor were composed of 3-mm stainless steel rods that were spaced 1.3 cm apart. The top was left open to enable EEG recordings in the freely moving rat. Two speakers were mounted to the aluminium left side wall. These speakers were used for presenting a 6-kHz tone with an intensity of 70 dB(A). The stimulus had a duration of 1 s with 10 ms rise and fall times. Each box was enclosed in a sound-attenuating chamber containing a printed circuit board, a set of cables, and a swivel for EEG measurements.

### **Electrode placement**

#### *Humans*

Silver EEG electrodes (Sensormedics) were placed at the Fz, Cz, and Pz sites according to the international 10-20 system, with the right mastoid as reference. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Horizontal EOG recordings were made from the outer canthus of the right eye; vertical EOG recordings were performed using electrodes placed supra-orbital to the right eye. Impedance was less than 5 k $\Omega$  for all subjects. EEG and EOG were filtered between 0.016 and 500 Hz and sampled at 1024 Hz.

#### *Rats*

A tripolar EEG electrode was implanted epidurally under isoflurane inhalation anaesthesia. The active lead was inserted near the vertex (A -3,5 L -2,0 related to bregma) and it was referenced to the cerebellum. The ground was also placed on the cerebellum. Two screws and dental acrylic cement were employed to fix the electrode on the skull surface. EEG was filtered between 0.1 and 500 Hz and sampled at 1024 Hz. Animals were allowed to recover from surgery for at least two weeks.

### **Procedure**

#### *Humans*

The experiment was run individually for each subject. The subject received instructions about the duration of the experiment, which was 27 minutes, but was not informed of the purpose of the experiment. Subsequently, the participant was seated in an armchair and was asked to sit as still as possible during the

experiment, with the eyes open. During the 27-min session, the 1-s tone was presented 100 times in four blocks of 25 presentations each. The inter-stimulus interval varied randomly between 5 and 10 s. The inter-block interval was 5 min.

#### *Rats*

First, the tripolar EEG electrode was implanted epidurally and the rats were allowed to recover for two weeks. On the day before the experiment, the rats were habituated to the operant boxes for 30 minutes. The experiment was run for eight rats at the same time. Session duration, tone presentation, and inter-stimulus and inter-block intervals were as described for the human participants.

## **Analysis**

#### *Wavelet denoising*

In order to be able to observe amplitude changes between single trials, a recently proposed method that is based on the wavelet transform, wavelet denoising, was used. In recent years, this wavelet transform was introduced to improve the time-frequency resolution of signal decomposition (Grossmann & Morlet, 1984). This is especially important in the case of ERPs, where interesting activity usually takes place in a fraction of a second and involves different ranges of frequencies (Quian Quiroga, 1998; Quian Quiroga, 2000; Quian Quiroga & Schürmann, 1999). Moreover, since each window contains only a few oscillations, stationarity of the signal is not necessary. The method significantly improves the visualization of single-trial ERPs in comparison with the raw data and also in comparison to previous approaches based on Fourier filtering (Quian Quiroga & Garcia, 2003).

#### *Component definitions*

The ERP components after denoising were determined on the basis of the single trial ERPs and defined as the maximum positive (P1, P2, P3) or minimum negative (N1, N2) values relative to a 200 ms pre-stimulus baseline. Table 1 summarises the human and rat components that were determined for statistical analysis.

*Table 1.* ERP components with corresponding latency ranges for the human and rat subjects

Component	Humans	Rats
P1	P55 (30-80 ms)	P30 (15-45 ms)
N1	N135 (70-200 ms)	N70 (40-100 ms)
P2	P200 (150-250 ms)	P115 (80-150 ms)
N2	N275 (240-310 ms)	N195 (140-250 ms)

### *Statistical analysis*

Only Cz data were used in the statistical analysis. The trials that were confounded by an eye-blink in the human subjects were not corrected or excluded from analysis. However, the data of the participants that frequently blinked their eyes during the trials in the experiment were excluded from statistical analysis. Furthermore, the number of levels within a block was reduced to six to minimize the effects of eye blinks and for reasons of statistical simplicity. The trials within a block were assembled, giving more weight to the first trials than to the last ones (Sambeth, Maes, Quijan Quiroga, Van Rijn, & Coenen, 2004). The following trials were analyzed: Trial 1 (Test Trial 1), Trial 2 (Test Trial 2), the means of Trials 3-4 (Test Trial 3), Trials 5-8 (Test Trial 4), Trials 9-16 (Test Trial 5), and of Trials 17-25 (Test Trial 6). The terms in parenthesis refer to the expression used to indicate these trials in the next sections.

Univariate (ANOVA) analyses of variance were performed using the data of both the human and the rat subjects. Separate analyses were performed for each ERP component with Test Trial (6 levels) and Block (4 levels) as within-subject factors. The Bonferroni test was used for post-hoc analyses. The level of significance was set at .05 throughout.

## **Results**

### **Visual inspection**

Six human participants and fifteen rats were excluded from the data analysis. These subjects were excluded because of, respectively, excessive eye-blinking and of the lack of a clear averaged ERP on at least one of the four 25-trial blocks. Figure 1 shows single trial ERPs of the six Test Trials of Block 1 for both humans and rats, respectively. Five ERP components were discerned for the human participants: the P1, N1, P2, N2, and P3 components. For the rat subjects, only

four components with the same order of polarity and an earlier latency were found: the P1, N1, P2, and N2 components.

It can be seen that the amplitudes of several components were larger in response to the first tone presentation compared to the other five tone presentations within a block (see Figures 2 and 3). Furthermore, the amplitudes of several ERP components decreased across the four blocks of stimuli, as can be seen in Figure 4.

Only the ERP components that the human and rat subjects had in common, that is, the P1, N1, P2, and N2 components, were analysed in this experiment. The results of the human P3 component are described in Sambeth et al. (2004). A summary of the significant effects is shown in Table 2.

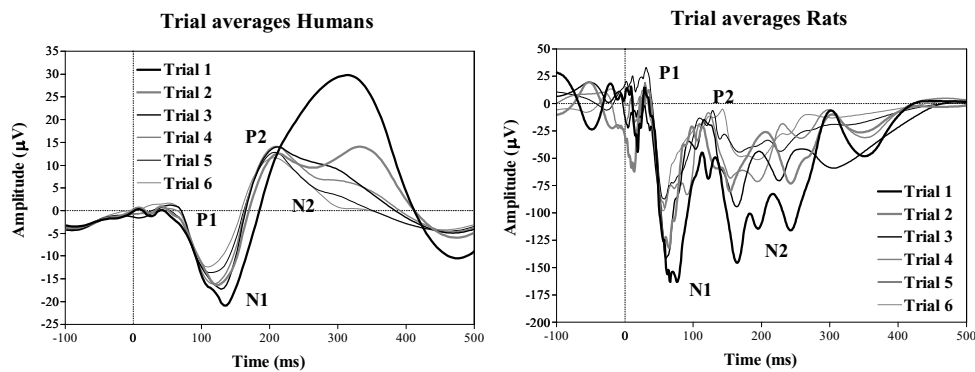


Figure 1. Single trial ERPs of the six weighted trials of Block 1 for both humans and rats (For the weighting procedure, see the statistical analysis section). Latencies are shown on the x-axis in milliseconds and amplitudes are presented on the y-axis in microvolts. Note that the amplitude of the N1 component in humans is larger on Trial 1 compared to the other trials. In rats, the N1 and N2 components are more negative on Trial 1 compared to Trials 2-6.

### Statistical analyses of human ERPs

An ANOVA on the amplitude of the P1 component did not reveal any significant effects ( $F = 1.56$ , .99, and .32, for Test Trial, Block, and interaction, respectively), as shown in Figure 2. The ANOVA on the amplitude of the N1 revealed an effect of Test Trial,  $F(5, 205) = 14.72$ ,  $p < .001$ , and Block,  $F(3, 123) = 2.99$ ,  $p = .034$ , but no interaction ( $F = .78$ ). Whereas post-hoc analysis revealed no significant differences between blocks, the post-hoc analysis on the factor Test Trial showed that the amplitude on Test Trial 1 was more negative than that on

Test Trials 3 to 6, and that the amplitude on Test Trial 2 was more negative than that on Test Trial 6, with the mean amplitudes (with standard deviations) being 29.6 (14.4), 24.9 (14.3), 23.8 (10.8), 22.8 (8.9), 22.6 (7.4), and 21.3 (6.6) for Test Trials 1, 2, 3, 4, 5, and 6, respectively.

The analysis on the P2 component revealed a significant main effect of Test Trial,  $F(5, 205) = 4.93$ ,  $p = .002$ , as can be seen in Figure 2. The amplitude was more positive on Test Trials 1, 2, and 4 than on Test Trial 6, with the mean amplitudes in microvolts (with standard deviations) being 23.5 (15.5), 27.8 (16.1), 19.7 (7.7), and 18.5 (7.5) for Test Trials 1, 2, 4, and 6, respectively. The main effect of Block and the interaction did not reach significance ( $F = 2.34$  and  $1.30$ , respectively).

## Humans

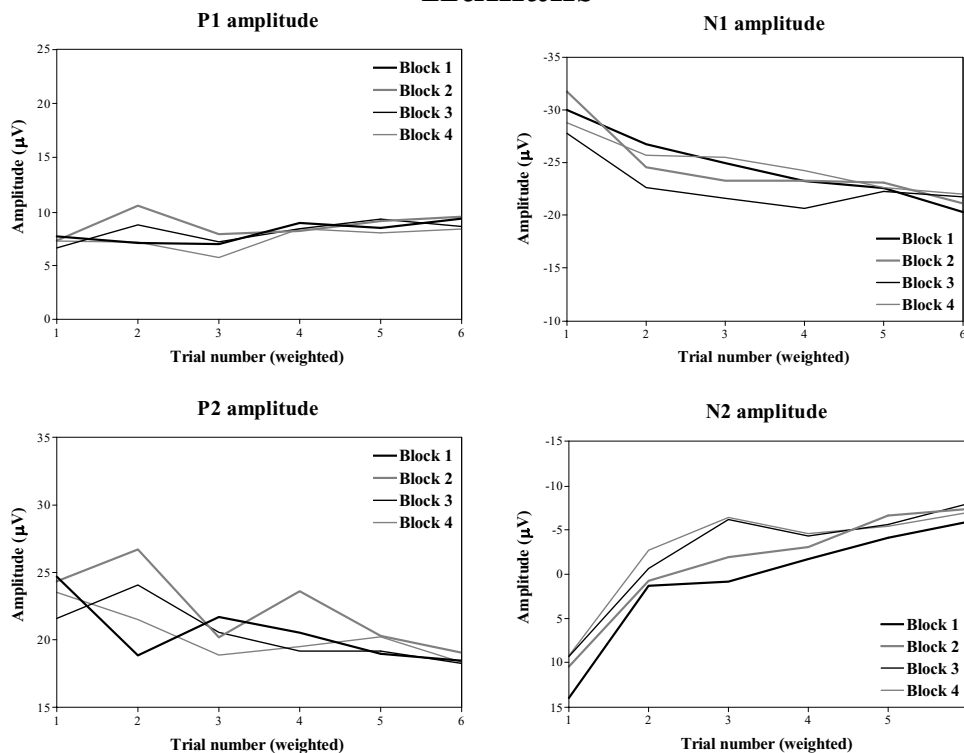


Figure 2. Maximum amplitudes of the P1, N1, P2, and N2 components of the human participants for the six weighted trials. The x-axis shows the trials; the y-axis the amplitude. Note that the amplitude of the N1 component becomes less negative from Trial 1 to Trial 6. The amplitudes of the N2 and P3a component become less positive with increasing stimulus presentations.

## Rats

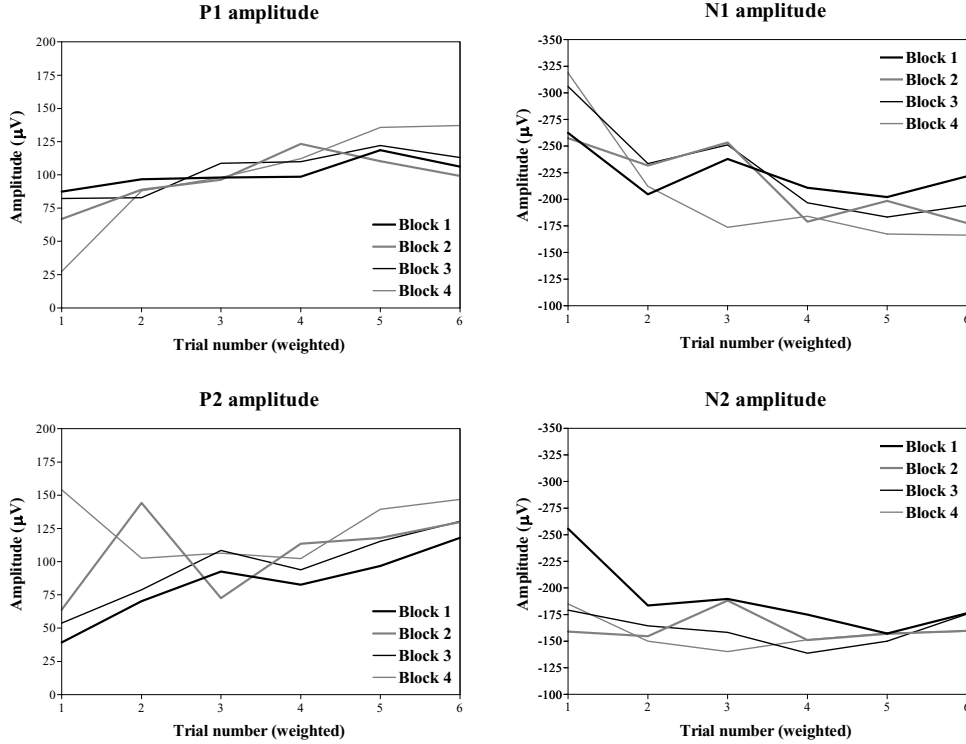


Figure 3. Maximum amplitudes of the P1, N1, P2, and N2 components of the rat subjects for the six weighted trials. The x-axis shows the trials; the y-axis the amplitude. Note that the amplitudes of the N1, P2, and N2 component become more positive from the first to the last trial.

The ANOVA on the amplitude of the N2 component showed a main effect of Test Trial,  $F(5, 205) = 51.18$ ,  $p < .001$ , and Block,  $F(3, 123) = 5.73$ ,  $p = .001$  ( $F = .82$  for the interaction). Post-hoc analysis revealed that the amplitude of this component was more positive on Test Trial 1 than on Test Trials 2 to 6, more positive on Test Trial 2 compared to Test Trials 5 and 6, and more positive on Test Trial 4 than on Test Trial 6, with the mean amplitudes being 10.7 (14.6), -0.3 (12.5), -3.4 (10.9), -3.4 (9.6), -5.4 (6.8), and -7.0 (6.8) for Test Trials 1, 2, 3, 4, 5, and 6, respectively. Post-hoc analysis further revealed that the amplitude was more positive in Block 1 compared to Blocks 3 and 4, with the mean amplitudes for Blocks 1, 3, and 4 being 0.7 (9.7), -1.3 (9.1), and -2.8 (11.3), respectively.

### Statistical analyses of rat ERPs

An ANOVA on the amplitude of the P1 component, as displayed in Figure 3, showed a main effect of Test Trial,  $F(5, 80) = 4.25$ ,  $p = .005$  ( $F = .08$  and  $.70$ , for Block and interaction, respectively). Post-hoc analysis revealed that the amplitude was more positive on Test Trials 5 and 6 compared to Test Trials 1 and 2, with the mean amplitudes in microvolts (with standard deviation) for Test Trials 1, 2, 5, and 6 being 66.0 (115.3), 89.2 (91.1), 121.8 (49.7), and 113.9 (52.5), respectively.

The analysis on the N1 component revealed a main effect of Test Trial,  $F(5, 80) = 14.56$ ,  $p < .001$ , which can be seen in Figure 3, but no Block effect or interaction ( $F = .72$  and  $1.03$ , respectively). The amplitude became less negative from Test Trial 1 to Test Trial 3-6 and from Test Trial 3 to Test Trial 5. The mean amplitudes of the N1 component for the Test Trials 1, 3, 4, 5, and 6 were -286.2 (144.5), -229.1 (110.5), -192.6 (72.3), -187.9 (61.5), and -189.9 (71.2), respectively. The ANOVA on the amplitude of the P2 component showed an effect of Test Trial,  $F(5, 80) = 4.05$ ,  $p = .002$ , and Block,  $F(3, 48) = 6.16$ ,  $p = .002$ , but no interaction ( $F = 1.74$ ). Post-hoc analysis revealed that the amplitude increased from Test Trial 1 to Test Trial 6 (amplitudes of 77.7 [86.3] and 131.3 [57.8], respectively) and the amplitude was more positive in Block 4 than in Block 1 (amplitudes of 125.3 [87.3] and 83.3 [71.2], respectively).

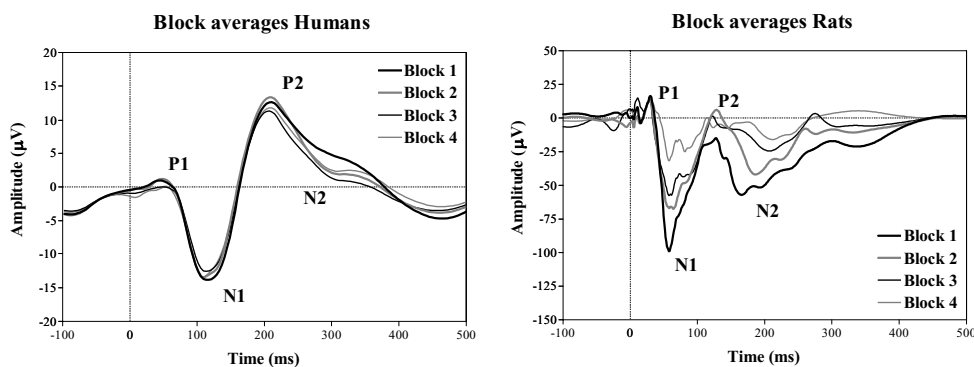


Figure 4. Averaged ERPs pooled over trials for both humans and rats. Latencies are shown on the x-axis in milliseconds and amplitudes are presented on the y-axis in microvolts. Note that the amplitude of the N2 component becomes less positive from Block 1 to Block 4 in humans, whereas the amplitude of the N1, P2, and N2 components becomes less negative in the course of the experiment in rats.

An ANOVA on the amplitude of the N2 component revealed a main effect of Test Trial,  $F(5, 80) = 2.94$ ,  $p = .021$ , and Block,  $F(3, 48) = 5.98$ ,  $p = .002$  (other  $F = .99$ ). Post-hoc analysis on the factor Test Trial did not show any significant effects, but the amplitude of this component was more negative in Block 1 compared to Blocks 2-4, with mean amplitudes of -189.7 (65.4), -161.7 (69.1), -161.1 (80.0), and -157.3 (77.2) for Blocks 1, 2, 3, and 4, respectively.

Table 2. Summary of significant results for both humans and rats

	Humans		Rats	
	Within blocks	Between blocks	Within blocks	Between blocks
P1	-	-	$F = 4.25^{**}$	-
N1	$F = 14.72^{***}$	$F = 2.99^*$	$F = 14.56^{***}$	-
P2	$F = 4.93^{**}$	-	$F = 4.05^{**}$	$F = 6.16^{**}$
N2	$F = 51.18^{***}$	$F = 5.73^{**}$	$F = 2.94^*$	$F = 5.98^{**}$

\*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p < .001$

## Discussion

The present study assessed to what extent the changes in amplitude of the human and rat ERP components in the course of a passive listening task are comparable. The auditory ERPs elicited by the stimuli in this passive task were characterized by the presence of two positive and two negative waves in both humans and rats, albeit with different latencies in the two species (P1, N1, P2, and N2).

The amplitudes of the human N1, P2, and N2 components and those of the rat P1, N1, and P2 components changed within a block (Test Trial effects). All these components, except for the rat P1, became closer to baseline (0  $\mu$ V). The rat P1 became more positive. Effects across the four blocks were found for the N2 component in humans and for the P2 and N2 components in rats. The amplitudes of these components became closer to baseline (0  $\mu$ V) in the course of the experiment.

It must be noted that it is not clear which of the rat ERP components are the equivalents of the human ERP components, as no studies have dealt with this issue before, at least not for the rat P1, N1, P2, and N2 components. Therefore, we cannot conclude whether the amplitude reductions were entirely comparable between the two species. However, in both species, the order of polarity of the ERP components was similar, although the latencies of the components were



shorter in the rats than in the humans. Furthermore, the amplitudes of the relatively early ERP components changed within a block of stimuli (the human N1, P2, and N2; the rat N1 and P2), whereas the late ERP components decreased across blocks of stimuli (the human N2; the rat P2 and N2). This suggests a correspondence between the ERPs of humans and rats.

The amplitude decrements of the ERP of both humans and rats within a block may reflect short-term habituation. In addition, it is possible that a recovery cycle affected the amplitudes of the human and rat ERP components or interacted with short-term habituation. The recovery cycle, that can be as long as 5 s for rat ERP components (De Bruin et al., 2001) and as long as 10-20 s for humans (Näätänen & Picton, 1987), might cause response decrements in itself or interact with 'real' habituation effects, because it decreases amplitudes due to sensory fatigue instead of learning. Particularly the early P1 and N1 components can be affected by a recovery cycle (Budd, Barry, Gordon, Rennie, & Michie, 1998; Carrillo-de-la-Pena & Garcia-Larrea, 1999; De Bruin et al., 2001).

Direct evidence for long-term habituation may be obtained when observing response recovery and enhanced re-habituation (Thompson & Spencer, 1966). If multiple sessions of trial blocks are presented, spontaneous recovery appears at the beginning of a new session or block. This means that the response at least partially recovers. Figures 2 and 3 show that such recovery was consistently the case across each block for all human ERP components and for the rat P1 and N1 components. Furthermore, in case of habituation, the response decreases faster as in the previous session or block during the stimulus presentations following the first stimulus. This phenomenon is called enhanced re-habituation. The human P3 component that was elicited in this study and which is described elsewhere (Sambeth et al., 2004) exactly showed this enhanced re-habituation. The amplitude of the P3 component decreased faster in the third and fourth block compared to Block 1. This suggests that the procedure used in this study elicits habituation. Therefore, it is likely that the amplitude reductions that we found across blocks in both humans and rats reflect long-term habituation.

In conclusion, the amplitude decrements found for the human and rat ERP components suggest a correspondence of the ERP correlates of elemental stimulus processing of humans and rats. In both species, these amplitude reductions may reflect habituation and / or recovery cycle processes.

## **Chapter 3**

# **Enhanced re-habituation of the orienting response of the human event-related potential**

A. Sambeth, J. H. R. Maes, R. Quian Quiroga, C. M. Van Rijn, and A. M. L. Coenen

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### **Abstract**

Previous studies found the amplitude of the orienting response (OR) of the human event-related potential to decrease with repeated stimulus presentations. This decrease has been suggested to either reflect short-term habituation and/or long-term habituation, both of which are learning processes. However, this earlier research failed to provide direct evidence supporting this claim. The present study attempted to show that the OR pattern shares one important feature of habituation: an enhanced response decrement across stimulus-presentation blocks (enhanced re-habituation). Participants received four blocks of 25 auditory stimulus presentations and showed an OR decrement both within (short-term habituation) and across (long-term habituation) blocks. Importantly, the OR decreased more rapidly during later than initial trial blocks, suggesting enhanced re-habituation. The latter result supports the notion that the amplitude decrement reflects an elementary learning process.

Descriptors: Habituation, enhanced re-habituation, orienting response, P3a, ERP, wavelet, single-trial

Habituation is a reduction in responding to a stimulus with repeated stimulus presentations and has frequently been demonstrated for peripheral, autonomic responses such as heart rate and galvanic skin response (e.g., Bradley, Lang, & Cuthbert, 1993). Habituation is considered to reflect an elementary learning process if it is not caused by factors like fatigue, a refractory period, or damage to the sensory system (Thompson & Spencer, 1966). It can be fast and only temporary (short-term habituation, STH), which is usually observed when stimuli are presented with short inter-stimulus intervals. Additionally, habituation may develop slowly, be relatively long-lived (long-term habituation, LTH) and is found at longer inter-stimulus intervals.

Evidence that this decreasing responsiveness indeed reflects an elementary learning process can be obtained using dishabituation procedures. In these procedures, the 'habituated' stimulus is either presented along with a novel stimulus, or slightly changed, or the context in which the stimulus occurs is altered. These manipulations increase responding to the habituated stimulus (Thompson & Spencer, 1966).

Spontaneous recovery and enhanced re-habituation are two further phenomena supporting an interpretation of STH and LTH in terms of a learning process. Specifically, if multiple habituation sessions are presented, the response will first recover to some extent at the onset of a new session (spontaneous recovery), and subsequently habituate to a larger extent and more rapidly within each following session (enhanced re-habituation) (Thompson & Spencer, 1966; Waters & McDonald, 1976).

Decrements in responding to a stimulus with repeated presentations have also been observed for a more central measure, the human event-related potential (ERP). One of the ERP components studied with repeated stimulations is the human orienting response (OR) or P3a component. This component reflects an orienting reaction in response to novel stimuli (e.g., Picton, 1992), may be found in passive listening tasks, and is most positive at frontal to central electrode sites. The more parietally elicited positivity is called the P3b and this component occurs in more complex tasks (e.g., Picton, 1992). In our experiment, we specifically focus on the OR/P3a component, most profoundly occurring at Cz.

Both short-term and long-term effects of the OR/P3a in response to repeated stimulus presentations have been studied. The OR component has shown amplitude reductions with repeated stimulus presentations within a passive listening task (Bourbon, Will, Gary, & Papanicolaou, 1987) and a passive oddball task (Polich & McIsaac, 1994). Furthermore, the OR also shows long-term

amplitude decrements in relatively passive paradigms (Pan, Takeshita, & Morimoto, 2000; Polich, 1989).

The short-term and long-term amplitude decrements that have been found for the OR/P3a component have generally been called 'habituation'. Although the reduction of the more autonomic responses such as heart rate indeed have been shown to reflect habituation (e.g., Waters & McDonald, 1976), to our knowledge, no direct evidence has been collected until now that response reductions that may be observed in ERPs with repeated stimulus presentations reflect habituation. The purpose of the present study, therefore, was to assess whether decreased responding, as reflected in the OR of the human ERP, shows one important feature of habituation, namely enhanced re-habituation. If so, this would constitute an important piece of evidence in favor of the claim that the previously observed OR reductions as a result of repeated stimulation indeed reflect habituation.

## **Method**

### **Subjects**

Forty-eight healthy students (12 men and 36 women, mean age 22 years) from the University of Nijmegen, The Netherlands, participated in the experiment. They received course credits. The participants were only allowed to take part in the study if they were healthy, did not use medication, and had no psychiatric history. Students who agreed to participate signed an informed consent.

### **Stimuli, electrode placement, and procedure**

The participants were tested in a sound-attenuating cubicle. A speaker used for presenting auditory stimuli was located to the right of the participant. The auditory stimulus used consisted of a 1-s 1500-Hz, 70-dB pure tone with a 10-ms rise and fall time. Recording of the EEG and the presentation of stimuli were controlled by a standard personal computer. The present experiment was part of a larger study performed at the University of Nijmegen. For reasons unrelated to the present experiment, the cubicle was illuminated for half the participants and dark for the other. As the statistical analysis showed no main effect of illumination, the pooled data of these participants will be reported in this paper.

Silver EEG electrodes (Sensormedics) were placed at the Fz, Cz, and Pz sites (10-20 system), with the right mastoid as reference. Horizontal and vertical eye movements (EOG) were detected from the right eye. Impedance was less than 5 k $\Omega$  for all participants. EEG and EOG were filtered between 0.016 and 500 Hz and sampled at 1024 Hz.

The participant received instructions about the duration of the experiment, which was 27 minutes. The subject was not informed of the purpose of the experiment. Subsequently, the participant was seated in an armchair and was asked to sit as still as possible, with his/her eyes open, during the experiment. During the 27-min session, the 1-s tone was presented 100 times in four blocks of 25 presentations each. The inter-stimulus interval varied randomly between 5 and 10 s. The inter-block interval was 5 min.

### Data analysis

In order to determine the amplitude of the single-trials, we used “wavelet denoising”, a recently proposed method based on the wavelet transform. Wavelet denoising gives a time-varying filter with excellent resolutions both in time and frequency. This is especially important in the case of ERPs, where interesting activity usually takes place in a fraction of a second and involves different ranges of frequencies (Quian Quiroga, 2000). After a wavelet decomposition, filtering is done by reconstructing the signal using only those wavelet coefficients (each one corresponding to a particular time and frequency range) that are correlated with the ERPs and setting the rest to zero. The selection of these wavelet coefficients is based on the wavelet decomposition of the average ERP (see [Quian Quiroga & Garcia, 2003; Quian Quiroga, 2000; Quian Quiroga & Van Luijtelaar, 2002] for details). It has been shown that the method significantly improves the visualization of single-trial ERPs in comparison to the original data and in comparison to previous approaches (Quian Quiroga, 2000).

The amplitude of the OR/P3a component after denoising was defined as the maximum positive amplitude between 280 and 400 ms after stimulus onset.

The number of dependent variables within a block was reduced to six for reasons of statistical simplicity. Because, in case of short-term habituation, responding to the first trials within a block decreases more than does responding to trials later in a block (Thompson & Spencer, 1966), the trials within a block were assembled, giving more weight to the first trials than to the last ones. The following trials were analyzed: Trial 1 (Test Trial 1), Trial 2 (Test Trial 2), the

means of Trials 3-4 (Test Trial 3), Trials 5-8 (Test Trial 4), Trials 9-16 (Test Trial 5), and of Trials 17-25 (Test Trial 6). The terms in parenthesis refer to the term used to indicate these trials in the next sections. The authors are aware of the fact that averaged responses may be smaller in amplitude than single responses simply because of averaging. However, as can be seen in the upper panel of Figure 2, the ORs in response to, for example, the trials containing Test Trial 6 were all smaller than was the OR to Test Trial 1. This indicates that averaging was legitimate.

A univariate analysis of variance (ANOVA) was performed using the amplitude of the OR for each of the electrode sites separately, with Test Trial (6 levels) and Block (4 levels) as within-subject factors. Short-term habituation (STH) would be reflected in a main Test Trial effect. Long-term habituation (LTH) was evaluated on the basis of a between-block comparison of the mean amplitudes within a block and was indicated by a main Block effect. Enhanced re-habituation would be reflected in a significant Test Trial x Block interaction effect, e.g., reflecting a block effect for Test Trial 2-6, but not for Test Trial 1. The Bonferroni test was used for post-hoc analyses. The level of significance was set at .05 throughout. Six participants were excluded from analysis because of excessive eye-blinking.

## Results

A main effect of Test Trial was found in the ANOVA at Fz, ( $F(5, 205) = 30.242$ ,  $p < .001$ ), Cz ( $F(5, 205) = 144.01$ ,  $p < .001$ ), and Pz ( $F(5, 205) = 109.58$ ,  $p < .001$ ). Post-hoc analysis on this factor revealed that the OR/P3a of Test Trial 1 was more positive than that of Test Trials 2 to 6 at all leads. Because of space limitations, only the results for the Cz site are presented in the upper panel of Figure 1. The OR in response to Test Trial 2 was more positive than that of Test Trials 5 and 6 again at all electrode sites, Test Trial 3 was more positive than Test Trial 6 at Pz, and Test Trial 4 was larger than Test Trial 6 at Cz.

A main effect of Block was found at Cz ( $F(3, 123) = 3.44$ ,  $p = .019$ ) and Pz ( $F(3, 123) = 4.51$ ,  $p = .005$ ), but not at Fz ( $F(3, 123) = 1.52$ ). Post-hoc analysis revealed that the amplitude in Block 1 was more positive than that in Blocks 3 and 4 at Cz and Pz, which again can be seen for Cz in the lower panel of Figure 1.

A significant interaction between Test Trial and Block was found at Cz ( $F(15, 615) = 2.07$ ,  $p = .022$ ), and a marginally significant effect at Pz ( $F(15, 615) = 1.71$ ,  $p = .074$ ). No interaction was present at Fz ( $F(15, 615) = 1.30$ ). Post-hoc analysis

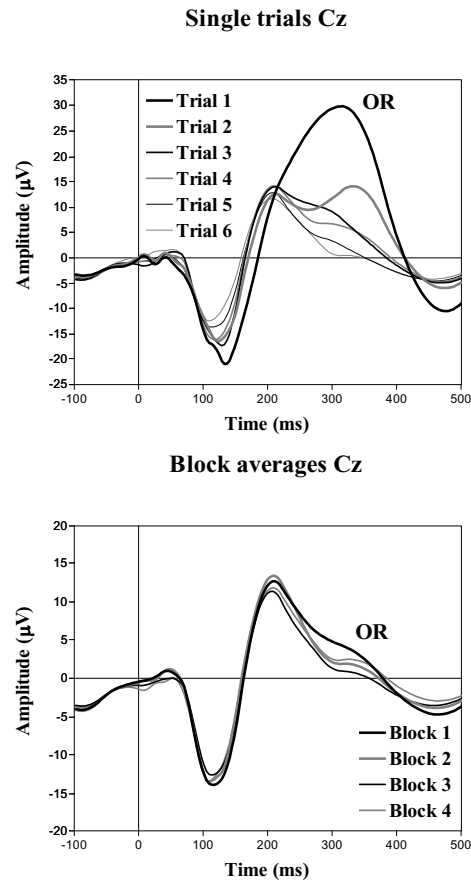


Figure 1. Upper panel: single-trial ERPs of the six Test Trials. Lower panel: averaged ERPs of the four blocks, pooled over trials. X-axes: time in milliseconds (ms). Y-axes: amplitude in microvolts (µV).

of the significant Test Trial x Block interaction at Cz revealed that Test Trial 3 was more positive in Block 1 than in Blocks 3 and 4. The amplitude of the OR at Test Trial 4 was more positive in Block 1 compared to Block 3. As Test Trial 1 did not differ between the different blocks, and as Test Trials 3 and 4 were larger in Block 1 than in Blocks 3 and 4, the amplitude in Blocks 3 and 4 decreased more compared to the amplitude in Block 1. This reflects enhanced re-habituation.

### Regression analysis

As the number of degrees of freedom in the ANOVA was large, a non-linear regression analysis of exponential decay was performed on the 25 Trials (see

upper panel of Figure 2) and the 6 Test Trials (see lower panel of Figure 2) separately for each block and each electrode site, in order to verify the interaction effect. The following formula was used:  $Y = (\text{Span}) * \exp(-K * (X-1)) + \text{Plateau}$  [Y being the amplitude, starts at Span + Plateau and decreases to 'Plateau' with a rate constant 'K'. Half span values are obtained after  $\ln 2/k$  Test Trials. X is the Test Trial.] The K-values were determined for each of the blocks for each participant separately. Next, the non-parametric Friedman test was performed on the K-values of the 6 Test Trials only, with the 4 blocks as test variables. In order to verify whether the results of the ANOVA, that is, larger amplitude decrements during Blocks 3 and 4 compared to Block 1, could be replicated, additional non-

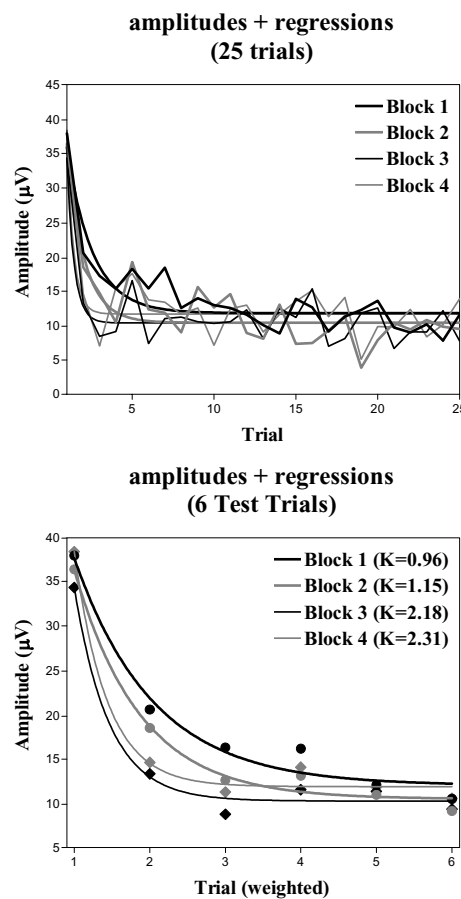


Figure 2.Upper panel: Maximum OR and regression lines of the 25 trials within each block. Lower panel: Regression lines and amplitude of the 6 Test Trials of the OR for each block. X-axes: the six trials. Y-axes: amplitude in microvolts ( $\mu\text{V}$ ).



parametric Wilcoxon rank order tests were performed.

The mean K-values per block are shown in the lower panel of Figure 2. The results of the non-parametric test revealed a marginally significant effect at the Fz ( $\chi^2 = 6.37$ ,  $p = .095$ ) and Cz sites ( $\chi^2 = 7.44$ ,  $p = .059$ ), but no effect at Pz ( $\chi^2 = 4.76$ ). Post-hoc Wilcoxon ranks tests showed that the K-values (the slopes) were larger for Block 4 than for Blocks 1 and 2 ( $p = .041$  and  $.004$ , respectively), and larger for Block 3 than for Block 2 ( $p = .011$ ), which can be seen in the lower panel of Figure 2 for Cz. This again indicates that the OR/P3a decreased more rapidly during the later blocks compared to the initial blocks.

## **Discussion**

The purpose of the present experiment was to assess whether the decrease in OR/P3a amplitude in humans subjected to repeated stimulus presentations is due to learning, or some non-learning process such as a refractory period or fatigue. This was studied by examining whether the OR amplitude decrease shows enhanced re-habituation. We first could replicate the short and more long-lasting decrements of the OR/P3a as was observed in other studies (Bourbon et al., 1987; Pan et al., 2000; Polich & McIsaac, 1994; Polich, 1989). More importantly, the amplitudes of the OR decreased more rapidly during Blocks 3 and 4 than during Blocks 1 and 2 at an expected electrode position (Cz), which implies enhanced re-habituation.

Former habituation studies (e.g., Waters & McDonald, 1976) used the number of trials needed to habituate as the measure of enhanced re-habituation, without showing learning curves, such as presented in Figure 2. We presented more relevant information, all providing evidence for enhanced re-habituation.

An argument against the notion of enhanced re-habituation reflecting fatigue or a refractory period is that the inter-stimulus interval we used was rather long. A refractory period only affects the ERP components at intervals shorter than 5 s (De Bruin et al., 2001). Furthermore, fatigue may develop with demanding tasks, whereas it does not develop if a task is boring (Smit, Eling, & Coenen, 2004), as was the case for the task in our study.

What does this habituation of the OR mean? According to the priming theory of Wagner (Wagner, 1976), an organism will be surprised at the presentation of a new stimulus. After the first presentation, a representation of this stimulus is placed into short-term memory. The next stimulus is primed, which leads to a larger expectation of that stimulus. This, in turn, decreases the response. This

hypothesis has been adopted to explain amplitude decrements in ERP research as well, since the amplitudes of ERP components decreased with increasing stimulus expectancy (Bourbon et al., 1987; Fruhstorfer, 1971). Accordingly, a large expectancy of the stimuli has caused the amplitudes of the OR/P3a to decrease in our experiment.

In conclusion, the present study supports the notion that amplitude reductions of the OR of the human ERP as a result of repeated stimulus presentation do reflect an elementary learning process, that is, habituation.



## **Chapter 4**

# **Auditory event-related potentials in humans and rats: effects of task manipulation**

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### **Abstract**

The purpose of this study was to compare components of the rat and human auditory event-related potential (ERP) as generated in active oddball and passive single-stimulus tasks. The rats were trained to discriminate between target and standard stimuli in an oddball task, while the human participants received instructions. Task effects on various ERP components were found in both species. Interestingly, effects on the P3 component were similar in the species with regard to amplitude: target stimuli elicited a higher amplitude in the oddball task than did standard stimuli. This might indicate that the P3 shares the same characteristics between species. However, the first four components occurred 1.82 times earlier in rats than in humans, expecting a P3 of about 200 ms in rats. The P3 in rats appeared at 380ms. We conclude that either the relation between human and rat peak latencies is not linear, or the P3 in rats is not the equivalent of the human P3.

Descriptors: Auditory event-related potential, P3, oddball task, human, rat

Event-related potentials (ERP) are averaged electroencephalographical potentials triggered by, and time-locked to, sensory stimuli (Näätänen, 1990). These potentials consist of various components that are either defined by polarity and order of occurrence (e.g., N2 is the second negative component), or by polarity and latency (e.g., P300 is a positive component approximately 300 ms after stimulus onset).

ERPs can be used to study information processes. Numerous ERP studies have been performed in humans, often in a so-called oddball paradigm. Frequently occurring standard stimuli are interspersed with infrequently occurring target stimuli, and subjects are instructed to count targets or to press a button after the presentation of the target stimulus. Both the N1, which is suggested to be involved in attention processes (García-Larrea, Lukaszewicz, & Mauguière, 1992; Näätänen, 1990), and the P3, which is involved in stimulus evaluation (Donchin, 1981), usually have a larger amplitude for target than for standard stimuli (Barrett, Neshige, & Shibasaki, 1987; Ochoa & Polich, 2000; Rockstroh et al., 1996). The latencies of the N1 and P3 are generally longer at target in comparison to standard stimuli or to stimuli in a passive paradigm requiring no response (Hirata & Lehmann, 1990; Mertens & Polich, 1997).

In addition, ERP studies have been performed in non-human species (Molnár, 1994). Monkeys (Glover, Onofri, Ghilardi, & Bodis-Wollner, 1986; Paller, McCarthy, Roessler, Allison, & Wood, 1992), cats (Başar-Eroglu, Başar, & Schmielau, 1991; Harrison, Buchwald, Kaga, Woolf, & Butcher, 1988) and rabbits (Wang, Shiraishi, Kawai, & Nakashima, 1998) all show a component in the cortical EEG that resembles the human P3 with respect to latency. Although a component (250-500 ms) that is somewhat similar to the human P3 with respect to latency has also been found in rats (Brankač, Seidenbecher, & Müller-Gärtner, 1996; Ehlers, Kaneko, Robledo, & Lopez, 1994; Hurlbut, Lubar, & Satterfield, 1987; Jodo, Takeuchi, & Kayama, 1995; Shinba, 1999), others proposed that an earlier component (220-240 ms) might be considered as the equivalent of the P3 in the rat (Yamaguchi, Globus, & Knight, 1993; Galicia et al., 2000). Latency alone, however, is not sufficient for suggesting equivalence of the P3 between rats and humans. Besides latencies, there are other uncertainties with respect to the P3 in rats. Much less work has been performed in rats than in humans. Its sensitivity to task manipulations is hardly described, and the earlier components preceding a P3 are not always reported (Brankač et al., 1996; Hurlbut et al., 1987; Jodo et al., 1995). This is important since amplitudes of earlier components might influence the amplitude of the P3.

A final point to bear in mind is the expected latency of components. As noted, some authors suggest that the latency of the P3 is the same in rats and humans (Brankačk et al., 1996; Ehlers et al., 1994; Shinba, 1999). However, the brain of rats is much smaller than that of humans. Therefore the conduction of a signal is faster, which should result in shorter latencies of components in rats compared to humans. This might imply that the P3 in rats, as found in some earlier studies, might not be the equivalent of the human P3 (Jodo et al., 1995; Shinba, 1997; Yamaguchi et al., 1993), but merely a reflection of some other late cognitive process.

Direct comparisons between the human and rat ERP were not made until now, although comparative studies form the basis for the search for the non-human equivalent of the human ERP, particularly the P3. These studies are important since such research may constitute a necessary basis for the establishment of neurophysiological substrata of cognitive processes. ERPs, as obtained in different species with an oddball paradigm, can be compared on similarities and differences in, for example, topographical localisation, influence of task manipulation and effects of drugs. One may have an argument for their equivalence if the components of the ERP in different species react in the same way in those species.

The present experiments assess the effects of task manipulation on the ERP of humans and rats. The subjects first received auditory target and standard stimuli in an active oddball task, and, subsequently, auditory stimuli in a passive single stimulus paradigm.

## **Method**

The present experiment was part of a larger study performed at the University of Nijmegen. The effects of a drug on the ERP were investigated in a double blind placebo controlled study in humans. In rats, silastic implants were used. Only the data of the placebo groups will be reported here.

## **Subjects**

### *Humans*

Fifteen students (6 males and 9 females, mean age 23 years) of the University of Nijmegen, The Netherlands, participated in the experiment. They were either paid for their participation, or received “research participation points” that the students have to collect as one of the study requirements. The participants were

medically examined before the experiment. Only healthy participants not using medication and without a psychiatric history were accepted. Participants who agreed to participate signed a written informed consent. The regional ethics committee approved of the project (CWOM, nr. 9809-0205).

### *Rats*

Twelve one-year old female Wistar rats served as the animal subjects. They were maintained on a 12-12 h light-dark cycle with lights off at 8.00 a.m. The animals were singly housed in Plexiglas cages in which they had unlimited access to water. The rats were kept at 85% of their free-feeding body weight ( $230 \pm 17\text{g}$ ) by restricted daily feeding, which is a very common procedure used in animal learning and memory studies in which food is used as a reinforcer (e.g. Maes & Vossen, 2001). Animals were handled daily during the experiment. The study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local ethics committee for animal experiments was obtained.

### **Apparatus**

The human participants were tested in a sound-attenuated, dimly-lit, cubicle (inside dimensions: 2 x 2.2 x 2 m). The subjects were seated in a comfortable chair. A small cross was painted on the wall 1.25 m in front of the chair, 1.10 m above the floor. A speaker was attached to the right side wall of the cubicle and was used for presenting a 1500 or 1750-Hz, 90 dB(A) tone. Participants had a button in their right hand during the experiment. Registrations of EEG, button presses and presentation of the tones were recorded and controlled by a standard personal computer.

The animal subjects were trained and tested in a set of eight identical operant boxes in which EEG recordings could be made. Each box measured 25 x 24 x 40 cm. The front and back walls were from clear Plexiglas; the right side wall and floor were composed of 3-mm stainless steel rods that were spaced 1.3 cm apart. The top was left open to enable EEG recordings in the freely moving rat. Centred in the aluminium left side wall was a 5 x 5 x 3 cm recessed food magazine to which 45-mg precision food pellets could be delivered. Visits to the food magazine were registered by means of an infrared emitter and sensor. Two speakers were mounted on the left side wall, 9 cm to the left and to the right of the food-magazine. These speakers were used for presenting a 6-kHz or a 10.5-kHz tone. The intensity of each tone was 78 dB(A). Each box was enclosed in a sound-

attenuating chamber containing a printed circuit board, a set of cables and a swivel for EEG measurements. Recording of the EEG and magazine visits, and the presentation of stimuli and food pellets were controlled by a standard personal computer.

## **Electrode placement**

### *Humans*

Silver EEG electrodes (Sensormedics) were placed at the Fz (frontal), Cz (central) and Pz (parietal) sites according to the international 10-20 system, with linked mastoids as reference. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Horizontal EOG recordings were made from the outer canthus of the right eye, vertical EOG recordings were done from electrodes placed infra and supra orbital to the right eye. Impedance was less than 5 k $\Omega$  for all participants. EEG and EOG were filtered between 0.16 and 100 Hz and sampled at 512 Hz. A 50 Hz notch filter was used off-line after data acquisition.

### *Rats*

A tripolar EEG electrode was implanted epidurally under isoflurane inhalation anaesthesia. The first active lead was inserted near the vertex (A  $-3,7$  L  $2,0$  related to bregma). The second active lead and the ground were placed on the cerebellum. Two screws and dental acrylic cement were employed to fix the electrode on the skull surface. EEG was filtered between 1 and 100 Hz and sampled at 512 Hz. A 50 Hz notch filter was used off-line after data acquisition.

Animals were allowed to recover from surgery for at least three weeks, in which they were offered food ad libitum. Following the recovery period, rats were implanted with empty silastic tubes subcutaneously (van Rijn, 1995). This procedure was a consequence of the fact that the rats served as a control group in a larger study assessing the effects of chronic diazepam on auditory ERPs. Subsequently, the rats were allowed to recover for three days. The food deprivation was reinstated following this period.



## Procedure

The experiment consisted of two tasks: an active oddball was followed by a passive single stimulus paradigm.

### *Humans*

400 stimuli were presented in the oddball task; 20% of these consisted of the target stimulus and 80% of the standard stimulus. For one half of the participants, the 1500 Hz tone served as the target, whereas the 1750 Hz tone was used as the target for the other half. Each stimulus presentation lasted 1000 ms; the rise-fall time was 10 ms. The inter-stimulus interval (ISI) was random between 3 and 5 seconds. The order of stimulus presentation was pseudo randomised, with the restriction that no more than one target stimulus was presented in succession. The participants were instructed to push the hand-held button after the target stimulus ended and to do this as quickly as possible. No response was to be made after the standard tone. Participants were given at least 10 practice trials with the stimuli prior to initiation of the actual experiment. A break of 5 minutes was given after this task.

Next, a total of 225 stimuli (referred to as passive stimuli) were presented in the passive stimulus paradigm. These stimuli consisted of only the standard stimulus used in the first task. The participants were asked to listen to the stimuli without having to respond.

Half of the subjects participated in the experiment in the morning and half in the afternoon. The participants had previously received instructions about the fact that they were only allowed to eat low fat food and were not allowed to drink coffee prior to the experiment. This instruction was necessary in view of the larger study this experiment was part of. The participants sat comfortably in their chair during the experiment and were instructed to keep their eyes focused on the cross. They were also asked to blink their eyes as little as possible and not to blink during stimulus presentations. Finally, they were instructed to sit as still as possible; when they had to change position, they were urged to do this in the ISIs.

### *Rats*

The animals first received two magazine training sessions (30 min.) in which they learned to retrieve food pellets from the food magazine. 10 food pellets were delivered according to a 3-min variable time schedule each session.

Subsequently, the rats were trained on a discrimination task in which they had to learn to respond to one of the two tones (the target stimulus) but not to the

other (the standard stimulus). Allocation of the 6 and 10.5 kHz tones to the target and standard stimulus was counterbalanced. Discrimination training was conducted in three stages. In Stage 1, each stimulus was presented for 30 seconds with an ISI of 3-5 minutes. A food pellet followed termination of the target stimulus; no food pellet was presented after the standard stimulus. For a total of 16 days, rats received a daily 1-hour training session consisting of a pseudo-random presentation of 6 target and 6 standard stimuli. The restriction was that no more than two trials in succession were of the same type. At the final session of Stage 1, all rats spent significantly more time in the pellet feeder during the target stimuli than during standard stimuli. In the subsequent phase, Stage 2, the stimulus duration was reduced to 15 seconds. This stage also lasted 16 days; further details were as in Stage 1. In Stage 3, which lasted 14 days, the stimulus duration was decreased to 5 seconds. Stage 3 was identical to Stage 2 in all other respects.

After training, EEG electrodes were implanted and the rats were allowed to recover for three weeks. Rats were subsequently retrained for 7 days on the same discrimination task as presented in Stage 3 (6 target and 6 standard stimuli in each session). Subsequently the silastic tubes were implanted and the rats were allowed to recover for three days. Finally, the actual test sessions were performed. For a period of 14 days, the rats received one 2.5-h EEG session each day. Seven target stimuli and 25 standard stimuli were presented within each session.

The passive single stimulus paradigm was initiated 3 days after the final oddball test session. A total of four sessions was presented, each consisting of 32 stimulus presentations. The stimulus used was the standard stimulus previously employed in the oddball task. The food magazine was not accessible during these sessions.

## **Data analysis**

### *Behavioural analysis*

Correct and incorrect responses were counted for the human participants. If subjects did not press the button during target stimuli, or if they pressed the button during standard stimuli, the response was to be considered incorrect.

The criteria for correct responding used in the analyses of the rat oddball data were at least one magazine visit in the time period of 200-5000 ms after onset of the target stimulus, and no single visit during the same interval at presentation of the standard stimulus.

*EEG analysis*

For humans, the EEG was visually checked off-line for EOG activity and other artifacts. The rat EEG was visually checked for movement artifacts. For both rats and humans, ERPs generated by stimuli that were presented in the presence of artifacts were excluded from further analysis. Also excluded were the ERPs associated with incorrect responding in the oddball task, that is, trials on which no response was made to a target stimulus, or on which a response was generated to a standard stimulus. The EEG fragments within an epoch of 100 ms before stimulus onset and 1000 ms after stimulus onset were averaged for all correct responses. The mean amplitude of the 100 ms before stimulus onset was used as a baseline value. The data of four rats were excluded because of poor quality EEG.

Separate averages for the three trial types, the target, standard and passive stimuli, were determined for each individual. Only the standard stimuli that occurred prior to a target stimulus were used in the analysis of the human oddball data, in order to have the same number of trials for target and standard stimuli. In the rat oddball task, the ERP evoked by all standard stimuli with a correct response was used so as to obtain a sufficient number of trials with a suitable EEG. Grand averages were constructed for trial types in the human and rat subjects.

The ERP components were determined on the basis of the individual and grand average ERPs, taking both the latency and amplitude variables into account. Table 1 summarises the human and rat components that were determined for statistical analysis.

*Statistical analysis of the EEG data*

Only Cz and Pz data were used in the statistical analysis, since the P3 is best visible at these locations (Lehmann, Michel, Pal, & Pascual-Marqui, 1994). Analysis of both the human and rat data revealed no effect of stimulus frequency (i.e., whether the target was the low or high-frequency tone). Therefore, the data were pooled across the (counterbalanced) stimulus frequency factor for both the human and rat behavioural and ERP data. Further analysis of the human ERP data revealed no differences between males and females on the different ERP components. For that reason, data were pooled across the factor sex.

Empty cells were present during the passive task in the rat study. Therefore, a conservative univariate analysis of variance (ANOVA) with stimulus type as between subject factor was employed for evaluating both the human and rat ERP data, in order to maintain comparability of results. All components were

separately analysed for amplitudes and latencies. The Bonferroni test was used for post-hoc analyses.

The human and rat ERP latencies were compared by means of a linear and a non-linear regression analysis on the latencies of the P1, N1, P2 and N2 components with additionally using the latency of the human P3 as a predictor for the latency of the P3 of the rat subjects.

*Table 1.* ERP Components with their corresponding latency ranges (after stimulus onset, in ms) for the human and rat subjects.

Component	Humans	Rats
P1	P50 (last positive before N100)	P20 (10-30 ms)
N1	N100 (90-150 ms)	N60 (41-80 ms)
P2	P200 (160-250 ms)	P120 (80-130 ms)
N2	N200 (240-350 ms)	N160 (130-200 ms)
P3	P300 (320-450 ms)	P380 (250-500 ms)

## Results

### Behavioural data

As only two human participants made errors (< 4 errors) in the oddball task, no statistical analysis was required to evaluate the human behavioural data. All human participants were clearly able to discriminate between target and standard stimuli.

The following analyses are based on the data from 8 rats. On the final session of discrimination training, the mean percentage of occurrences of at least one food magazine visit within the time window of 5 seconds was 97.9% for the target stimuli and 41.8% for the standard stimuli. An ANOVA with stimulus type (target vs. standard) as the single within-subjects factor on these data revealed a highly significant effect ( $F(1, 7) = 17.7, p < .01$ ), indicating reliable discrimination performance.

The performance during the test phase was as follows. Across all test sessions, the mean percentage of occurrences of at least one magazine visit was 97.1% and 23.5% during the target and standard stimulus presentations, respectively. An ANOVA on the data obtained during the test phase revealed a significant effect ( $F(1, 7) = 36.8, p < .001$ ). Rats were able to discriminate between the target and

standard stimuli after recovery from surgery and their performance even tended to increase.

## Human ERPs

Grand average ERPs of the Cz and Pz sites are shown in the upper two panels of Figure 1, and mean amplitudes and latencies of the ERP components are presented in Table 2. As can be seen in Figure 1, the amplitudes of the P3 component that were evoked by the target stimuli were larger than those for standard and passive stimuli. In addition, standard stimuli had higher amplitudes than had passive stimuli on this component. The amplitude of the N1 target and standard stimulus seemed to be more negative than that of the passive condition.

### Cz

The effect of stimulus type was significant for the N1 component amplitudes ( $F(2, 42) = 4.8, p < 0.05$ ). Post-hoc analysis revealed that N1 amplitude was significantly greater for target than for passive stimuli ( $p < 0.05$ ). An analysis on the amplitudes of the N2 also showed a main effect ( $F(2, 42) = 4.6, p < 0.05$ ), and subsequent analyses revealed that passive stimuli had significantly larger amplitudes ( $p < 0.05$ ) than did target stimuli. In addition there was an effect of stimulus type on the amplitudes of the P3 ( $F(2, 42) = 20.9, p < 0.001$ ). Target stimuli displayed higher amplitudes than did standard ( $p < 0.01$ ) and passive ( $p < 0.01$ ) stimuli on this component, and the amplitude of standards was higher than that of passive stimuli ( $p < 0.05$ ). A significant effect on latency was found for the P3 component ( $F(2, 42) = 4.8, p < 0.05$ ). Target stimuli had a longer latency than standard stimuli ( $p < 0.05$ ).

### Pz

Analyses of the Pz data revealed results similar to those of the Cz site data, with additional effects on the P1 and N2 latencies. The N1 component amplitudes showed significant effects ( $F(2, 42) = 3.9, p < 0.05$ ). Post-hoc analysis revealed that N1 amplitude was significantly higher for the target compared to the passive stimuli ( $p < 0.05$ ). In addition there was an effect on the amplitude of the N2 component ( $F(2, 42) = 10.8, p < 0.05$ ). Amplitudes were significantly more negative for the standard ( $p < 0.05$ ) and passive ( $p < 0.01$ ) stimuli than for the target stimuli. An analysis on the amplitudes of the P3 revealed a significant effect ( $F(2, 42) = 38.9, p < 0.001$ ). Target stimuli displayed larger amplitudes than standard ( $p < 0.01$ ) and passive ( $p < 0.01$ ) stimuli, and standard stimuli had

larger amplitudes than passive stimuli ( $p < 0.05$ ). Latency effects were found for the P1 ( $F(2, 42) = 6.2, p < 0.01$ ) and N2 ( $F(2, 42) = 9.7, p < 0.001$ ) components. P1 latency was longer for the target ( $p < 0.01$ ) and standard ( $p < 0.05$ ) stimuli than for the passive stimuli. The latency of the passive stimuli ( $p < 0.01$ ) was longer than that of the target stimuli on the N2 component.

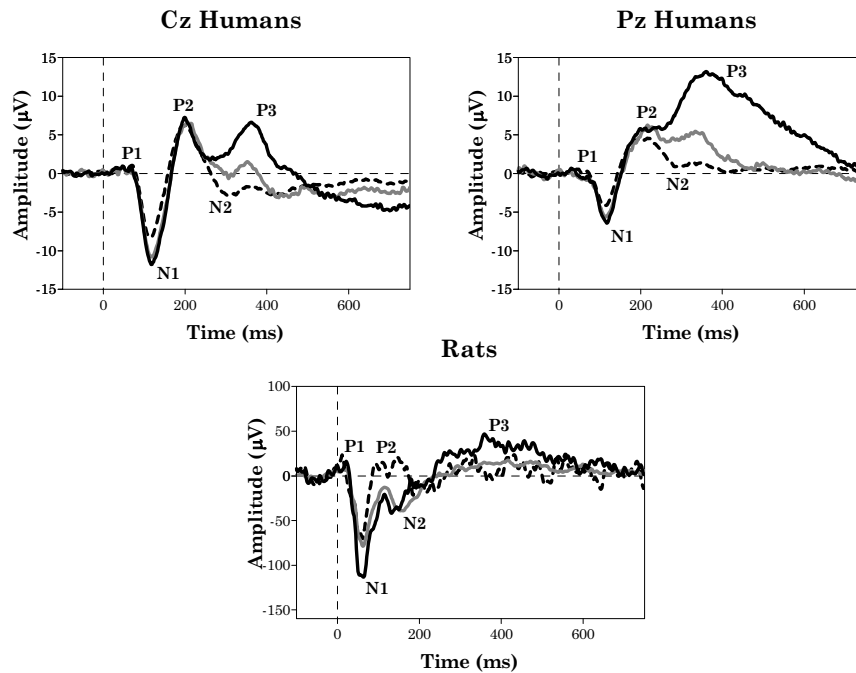


Figure 1. Human and rat grand average ERPs evoked by target (black line), standard (grey line) and passive (dotted line) stimuli. Latencies are shown on the x-axes in milliseconds and amplitudes are given on the y-axes in  $\mu\text{V}$ .

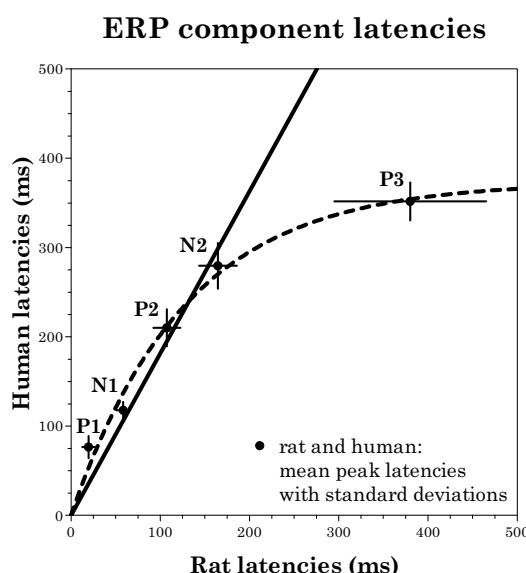
## Rat ERPs

Grand average ERPs of rat subjects are shown in the lower panel of Figure 1; mean amplitudes and latencies of the components are presented in Table 2. As can be seen, five components with the same order of polarities as found for the human ERPs were present, with latencies of 20, 60, 120, 160 and 380 ms, respectively. The averages for the target and standard stimuli are based on eight rats, whereas the average for the passive stimulus is based on the data of four rats only. The latter number of rats was lower than the former because of a loss of

an appropriate EEG signal for four rats at the time of performing the single stimulus task.

As can be seen in Figure 1, the target stimulus tended to have a greater amplitude of the N1 component than did the standard and passive stimuli. The P3 amplitude appeared to be more positive for target stimuli in comparison to the other two stimulus types.

Statistical analysis revealed a significant effect on the amplitude of the P3 component ( $F(2, 17) = 6.5, p < 0.01$ ). The amplitude of the P3 elicited by the target stimuli was higher than that of standard stimuli ( $p < .01$ ). None of the other analyses revealed significant effects.



*Figure 2.* Results of a linear (straight line) and non-linear ( $Y=Y_{\max}*(1-\exp(-K*X))$ ), (dotted line) regression analysis of the ERP components of human and rat subjects. The linear regression analysis was performed on the P1, N1, P2, and N2 components, the non-linear regression analysis was performed on all five components, including the P3. Mean latencies (in ms) with standard deviations of the five components are shown for the rat (x-axis) and human (y-axis) subjects.

### Between-species comparison of latencies

Figure 2 shows the mean latencies with SDs of the 5 components, of both rats and humans. Since the peak-to-peak amplitude relations of the first four components of the rat and human ERP show remarkable resemblance, we chose to perform regression analyses on the latencies of the components. Linear and

non-linear regression analyses were performed on the mean data. The linear regression was performed on the latency data of the first four components. These components occurred 1.82 times earlier in rats than in the human participants ( $r^2 > 0.98$ ). Assuming a linear relationship, on the basis of the latency of the human P3 component, the latency of the P3 component of the rats would be about 200 ms. However, the P3 component of rats was found to be 380 ms. Therefore, a linear relationship is not adequate to describe the data. A non-linear relationship ( $Y=Y_{\max}*(1-\exp(-K*X))$ ) with an  $Y_{\max}$  of 373 ms and a  $K$  value of  $7.8 \cdot 10^{-3}$  does describe all the components adequately ( $r^2 > 0.96$ ).

## **Discussion**

The aim of the present study was to evaluate and compare ERP components of rats and humans. The ERPs elicited in the oddball paradigm and passive condition in the two species were characterized by the presence of three positive and two negative components, albeit with different latencies in the two species. The amplitude of the P3 component showed similar task effects, whereas the effects on the N1 component differed.

The effects observed in the human subjects are discussed first. Effects on the amplitude of the N1 in the human oddball task were, for example, studied by Rockstroh et al. (1996). They reported larger amplitudes on the N1 component for target than for standard stimuli, an effect that was not obtained in our study. In our present human study, however, the target stimulus evoked a larger amplitude in comparison with the passive stimulus. In a task similar to ours, García-Larrea et al. (1992) found that standard stimuli had higher N1 amplitudes than did stimuli in a neutral, single-stimulus paradigm. Dissimilarities between the target and neutral stimuli were not investigated. They proposed that the standard stimulus is “cognitively evaluated”, or receives relatively more attention, whereas this is not the case for the neutral stimulus. The results of our study might also be explained by this cognitive evaluation.

Effects on the amplitude of the N2 and P3 components in humans were found in the present study. The effects on the N2 were reverse to the effects found on the N1 and P3; amplitudes of the target stimuli were less negative than those of standard and passive stimuli. A larger P3 amplitude was found for target stimuli than for standard and passive stimuli. Considering that the N2 is situated on the rising flank of the P3 component, the effect on the N2 and the P3 might be dependent.



Table 2. Mean amplitudes (in  $\mu V$ ) and latencies (in ms) of the ERP components of both humans and rats.

Component	Stimulus	Human participants Cz			Human participants Pz			Rat subjects		
		Amplitude (SD)	Latency (SD)		Amplitude (SD)	Latency (SD)		Amplitude (SD)	Latency (SD)	
P1	Target	1.7 (3.0)	69.6 (6.5)	-0.1 (3.4)	81.3 (12.3)	18.1 (18.6)	20.3 (4.7)			
	Standard	1.0 (2.4)	74.1 (10.5)	0.3 (2.3)	79.2 (12.4)	10.6 (20.0)	22.5 (6.0)			
	Passive	1.5 (1.9)	69.5 (7.2)	0.9 (1.5)	67.9 (8.2)	23.3 (20.7)	11.0 (0.0)			
N1	Target	-12.7 (4.2)	120.9 (10.2)	-7.2 (3.1)	122.3 (12.1)	-127.9 (34.9)	57.8 (6.7)			
	Standard	-11.9 (3.8)	119.5 (7.3)	-6.6 (2.8)	116.3 (6.7)	-89.8 (43.2)	60.5 (8.7)			
	Passive	-8.9 (2.4)	118.3 (9.7)	-4.5 (2.3)	114.5 (7.2)	-89.1 (23.4)	59.0 (8.9)			
P2	Target	8.1 (5.2)	199.9 (9.5)	8.1 (3.1)	209.3 (18.6)	-2.3 (58.7)	108.9 (17.2)			
	Standard	8.0 (4.9)	205.3 (12.2)	7.7 (3.4)	216.7 (19.8)	-5.7 (28.6)	110.9 (12.1)			
	Passive	7.5 (2.5)	199.3 (15.1)	6.0 (2.9)	203.9 (22.9)	29.4 (24.2)	100.3 (12.9)			
N2	Target	0.1 (3.6)	289.2 (21.3)	4.7 (2.8)	262.8 (24.9)	-50.0 (70.0)	154.4 (22.7)			
	Standard	-2.2 (3.7)	293.3 (18.4)	2.0 (3.4)	278.0 (18.6)	-49.1 (31.3)	167.4 (19.2)			
	Passive	-3.6 (2.6)	303.3 (19.2)	0.1 (1.7)	297.7 (21.4)	-27.9 (36.5)	176.8 (23.3)			
P3	Target	7.8 (4.8)	363.9 (13.1)	14.9 (5.3)	359.0 (22.4)	77.8 (35.3)	384.9 (83.2)			
	Standard	2.7 (3.7)	346.4 (17.2)	6.4 (4.0)	344.3 (17.0)	28.7 (9.0)	374.4 (91.7)			
	Passive	-1.2 (2.5)	357.1 (16.3)	2.4 (1.9)	351.7 (22.6)	51.5 (33.4)	382.0 (99.0)			

Ochoa and Polich (2000), and Rockstroh et al. (1996), and many others found, in humans, that the target stimulus elicited a higher amplitude of the P3 than did the standard stimulus. This is in full accordance with the present results. Moreover, the amplitude of the P3 for the standard was also higher than that for the passive stimulus. García-Larrea et al. (1992) did not find a P3 in the neutral, single-stimulus paradigm. However, they performed the single-stimulus task prior to the oddball task, whereas the reverse order was in effect in the present study. Consequently, the stimulus used in the present single-stimulus paradigm might be less “neutral” than was the case in García-Larrea et al.’s study. In our case, there might have been a carry-over effect from the previous oddball task. This difference might have been responsible for finding a (small) P3 component for the passive stimuli in the present study but not in theirs.

The P3 latency was longer for target stimuli than for standard stimuli in the current study. This effect is equivalent to that obtained in other experiments (e.g., Mertens & Polich, 1997; Rockstroh et al., 1996).

In all, it seems that the differences in components between target, standard, and neutral stimuli are in agreement with the outcomes reported by others. The present oddball task, which differs from the task typically used by others in several respects (e.g., longer stimulus duration, requirement to respond after cessation of the target instead of as quickly as possible after its onset), seems to elicit quite similar ERPs in comparison to the typical, traditional oddball task. The oddball paradigm as used in the human part of the study is perhaps more comparable to the oddball task used in the rat part of the study and may form a basis for interspecies comparisons.

Five components with the same order of polarity as found in the human study were detected in the ERP of the rats with latencies of 20, 60, 120, 160, and 380 ms, respectively. Notably, the latencies of the first four components of the rats were much shorter than were those of the first four components of the human ERP.

Analyses of the rat ERPs only revealed a task effect for the P3 component. This component was larger for target stimuli than for standard stimuli, which is in agreement with what can be expected in an oddball task.

The latencies of the P1, N1, P2, and N2 components of the rats found in the current study correspond to those observed by Shinba (1997), Ehlers et al. (1994), Yamaguchi et al. (1993), and Meeren, Van Cappellen van Walsum, Van Luijckelaar, and Coenen (2001). The latency of the P3, however, differs between studies. Some found a latency similar to that in the present study (Shinba, 1997, 1999; Ehlers et

al., 1994; Jodo et al., 1995; Hurlbut et al., 1987; Brankač et al., 1996), whereas others (Yamaguchi et al., 1993; Galicia et al., 2000) observed a shorter latency. Comparing the latency of the P3 component between various studies is rather difficult since the morphology of the P3 is not always as sharp as that of the earlier components, and also because standardization of localization of EEG electrodes and the choice of the reference is highly variable between various laboratories.

No significant effect was found for the amplitude of the N1, probably because of the large variability between subjects. Shinba (1997) and Galicia et al. (2000), on the other hand, did find effects. Ehlers et al. (1994) found a longer latency of the N1 for target stimuli in an active oddball than for target stimuli in a passive oddball, whereas the amplitude was not affected. The results of these animal studies thus seem to be contradictory. Effects on amplitude (this report; Barrett et al., 1987) and latency (Hirata & Lehmann, 1990) of the N1 are common in oddball tasks with human subjects, though. More research is needed to verify whether the rat N1 is actually influenced by this task manipulation and, subsequently, whether the rat N1 shares some characteristics with the human N1.

An effect was found for the amplitude of the P3: target stimuli had larger amplitudes than did standard stimuli. Several rat P3 studies obtained larger amplitudes for the P3 at target compared to standard stimuli (Ehlers et al., 1994; Jodo et al., 1995; Shinba, 1999; Yamaguchi et al., 1993). Most of them did not find a longer P3 latency for target than for standard stimuli (Ehlers et al., 1994; Jodo et al., 1995; Yamaguchi et al., 1993), which is in accordance with the current data. However, some authors found P3 latencies shorter than the latency in our study. Since an ERP is also defined by its latency, it should therefore be doubted whether the same P3 is described in these studies.

Some general issues must be kept in mind when making inter-species comparisons, before trying to actually compare the results of rats and humans. Rats not only have dissimilar auditory thresholds to humans, their sensitivity to high pitch tones is also higher than that for low pitch tones. Humans are insensitive to high pitch tones. This implies that appropriate human and animal tasks always differ with respect to the frequency of the stimuli used. This also holds for the current study, in which tones of higher frequency were used for the rats than for the human subjects.

A further comment regarding the frequency of tones is as follows. Knight, Brailowsky, Scabini and Simpson (1986) investigated the relationship between pitch and amplitudes of ERP components in rats. It was found that amplitudes

increase with an increase from 2 to 8 kHz and reach a plateau between 8 and 20 kHz. Previous rat ERP studies (Brankačk et al., 1996; Ehlers et al. 1994; Hurlbut et al., 1987) used frequencies of 4 kHz and lower. The target stimulus used in these studies was always the stimulus with the higher frequency. Therefore, task effects were confounded with stimulus frequency and this may have caused the pronounced differences between target and standard stimuli. In the present experiment, such confound was prevented by a counterbalanced design.

Another comment on rat ERP studies is related to the task in which rats are engaged. Convincing documentation of the behavioural responses is sometimes lacking in rat studies (Molnár, 1994), or the response to be made is not compatible with the rat's normal behavioural repertoire. For example, rats are sometimes head-restrained (Shinba, 1999). In the present study, rats were trained to visit a magazine in order to collect food in the presence of the target stimuli and not to respond upon presentation of the standard stimuli. As verbal instructions and feedback are impossible in animal studies, the choice of an appropriate behavioural response is an imperative (Molnár, 1994) and needs careful monitoring. If, in the studies performed until now, the rat P3 indeed is not comparable to the human P3, it might be argued that this is a consequence of the rat oddball tasks being fundamentally different from the human oddball tasks. In this respect, Ehlers et al. (1994) argued that rats are particularly motivated to perform the task in order to get food rewards, whereas this type of motivation is lacking in human subjects. Humans might be motivated for other reasons. Whether or not this really constitutes a fundamental difference between the two types of oddball tasks remains to be determined.

We now turn to the actual comparison of the human and rat the ERP components. The N1 component showed task effects for the human subjects. It elicited more negative amplitudes at target stimuli than at standard stimuli. Although the amplitudes of the target stimuli were more negative at target compared to standard stimuli in rats as well, this result did not reach statistical significance, probably because of substantial variability between subjects. It is therefore not clear whether the N1 component is comparable between the two species.

Several researchers have discussed the functional equivalence of the human P50 and the rat P13, P17, N22 or N40-50 component (e.g. Adler, Rose, & Freedman, 1986; Bickford-Wimer et al., 1990; Boutros, Bonnet, Millana, & Liu, 1997; Miyazato, Skinner, & Garcia-Rill, 1999; Miyazato, Skinner, Crews, Williams, & Garcia-Rill, 2000; De Bruin et al., 2001). The debate involves ERP

responses in the so-called sensory gating paradigm, in which two identical stimuli are presented with an interval of 500 ms, and ERP responses to the second stimulus are usually smaller than to the first. Some researchers (Miyazato et al., 1999, 2000) suggest that the rat P13 is the equivalent of the human P50. For example, both components respond similarly in the sensory gating paradigm during several sleep stages, both are affected by scopolamine, and both components have the same polarity. However, other results (Adler et al., 1986; Bickford-Wimer et al., 1990; Stevens, Fuller, & Rose, 1991; Adler, Hoffer, Griffith, Waldo, & Freedman, 1992; Boutros, Uretsky, Berntson, & Bornstein, 1994) are more in favour of the suggestion that the rat N40-50 is equivalent to the human P50, since other drugs (e.g. amphetamine, haloperidol, cocaine, and nicotine) changed the human P50 and rat N40-50 components comparably. De Bruin et al. (2001) showed that the vertex P17 and N22 are decreased with repetitive stimulation and interstimulus dependent, and suggested that these components are the most likely candidates for the rat homologue of the human P50. As no significant task effects were present in the current study for either the human P50 (our P1) or the rat P13 or P17 (our P1) and N22 or N40-50 (our N1) amplitudes, no clear arguments in favour of either of the points of view can be given, and more research on possible cognitive effects on these components will be necessary in the future.

One other component that did show effects in both species is the P3 component. In both humans and rats the amplitude of the P3 component was larger for target than for standard stimuli. The effects on the latency of this component, however, differed. Significant task effects were found in the human subjects, but not in the rats.

However, the suggestion that amplitudes of ERP components of the species do not react completely equally to task manipulations is preliminary, considering that only few studies were performed in rats. One of the criteria that are generally adopted for deciding whether the rat P3 matches the human P3 is the latency of the components (Hurlbut et al., 1987; Shinba, 1997; Yamaguchi et al., 1993). In some studies (e.g., Ehlers et al., 1994; Shinba, 1997; 1999), the P3 in rats occurs approximately 400 ms after stimulus onset, a result also found in the present study. Latencies in human studies are usually between 250 and 500 ms after stimulus onset (Ochoa and Polich, 2000). This similarity in latency between humans and rats suggests that the P3 component is comparable between those two species. However, this suggestion is compromised by the fact that the earlier components (P1, N1, P2, N2) occur about 1.8 times earlier in rats than in humans

(see Figure 2). Furthermore, whether a component is comparable between different species does not depend on the polarity of that component. The location of the source of a certain activity and the morphological properties of its surrounding tissue determine the cortical polarity of an ERP component. This could well differ between humans and rats. But, given the remarkable resemblance of the peak-to-peak amplitude relations, it might be tempting to compare the components in order of occurrence. If the temporal relation of ERP components of humans and rats would be comparable and linear, the latency of the P3 in rats would be about 200 ms (see Figure 2). On the other hand, when assuming a non-linear relation, one might indeed find a P3 component with a latency of about 370 ms in rats. This latency is quite similar to the latency of the human P3, and to the actual results found for the rats in the present study. It might be suggested that the first four components of the rat ERP, which occur 1.8 times earlier than the human components, depend more on sensory processes, whereas the later P3 component is dependent on more elaborative processing of stimuli. Whether or not this processing is equivalent between the two species cannot clearly be concluded by our regression results. An assumption that can be made, however, is that this component does reflect some kind of late cognitive effect. In the rats, its amplitude was enhanced when the presentation of the stimulus was associated with the presentation of food.

In conclusion, the present study compared the ERPs of rats and humans. Results showed some similarities and dissimilarities between the ERP components of both species. More research adopting a strategy similar to that used in the current study, like investigating the effect of drugs on the ERP in an oddball study, or exploring the topographical distribution, is however needed, since compelling evidence for inter-species comparability of ERP components remains scarce.



## **Chapter 5**

# **Effects of learning on the event-related potential in an oddball task**

A. Sambeth, J. H. R. Maes, A. M. L. Coenen, and J. Brankačk

### **Abstract**

This study aimed at assessing the effects of learning on event-related potential (ERP) components in an oddball task. Human participants learned to press a button in response to target, but not standard stimuli. The N100 and P300 components, evaluated at Fz, Cz, and Pz, were equally large at the start when the participants had not yet learned that the target was the relevant stimulus, whereas they were larger in response to target than to standard stimuli at the end of the experiment. The results indicate that learning affects the ERP components in humans.

Descriptors: event-related potential, human, learning, oddball task, P300



Event-related potentials (ERP) are often elicited in so-called oddball tasks. In these tasks, frequent standard stimuli are interspersed with infrequent targets and the subjects are instructed to press a button in response to the targets. In humans, the N100 and P300 components are enlarged at target presentations compared to presentations of the standard stimulus (e.g., Katayama & Polich, 1996; Ochoa & Polich, 2000; Rockstroh et al., 1996; Sambeth et al., 2003).

The human N100 is said to reflect attention processes (e.g., Kok, 1997; Näätänen & Picton), whereas the P300 component is affected by factors such as task relevance, task complexity, or target probability (e.g., Johnson, 1986; Katayama & Polich, 1996; Kok, 2002).

So far, only the ERP components to correct responses in the oddball task, that is, a response to the target and no response to the standard stimulus, were examined. As it is common to instruct human subjects about the task requirements, in practice all stimuli can be analyzed in those experiments. The effects of learning on ERP components as elicited in an oddball task have, however, never been studied in humans before. Studying these effects may give us further insight into the electrophysiological correlates of learning and cognitive processes. Therefore, the present experiments aimed at examining the effects of learning on the human ERP components during an oddball task. The participants in this study learned to press a button after a target stimulus, but not after a standard stimulus. The ERPs were analyzed in response to both the first and the last twenty target and standard presentations.

## **Method**

### **Subjects and electrode placement**

Eighteen students (four men, mean age 22 years) of the University of Nijmegen, The Netherlands, participated in the experiment. They were either paid for their participation, or received course credits. They were only allowed to take part in the study if they were healthy, did not use medication, and had no psychiatric history. Subjects who agreed to participate signed a written informed consent.

EEG activity was recorded using an electrode cap with tin leads from the Fz, Cz, and Pz sites according to the international 10-20 system, with the right mastoid as reference. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Impedance was less than 5 k $\Omega$  for all

participants. EEG and EOG were filtered between 0.016 and 100 Hz and sampled at 512 Hz.

### **Stimuli and procedure**

First, EEG and EOG electrodes were attached to the skin. Next, the participants performed an auditory oddball task. Two auditory tones were used as stimuli, a 1000 Hz tone that served as target and a 2000 Hz tone that served as a standard. Both stimuli had a duration of 50 ms, with 10 ms rise and fall times and an intensity of 70 dB.

The participants were presented with a total of 60 target (probability 20%) and 240 standard stimuli, divided over three blocks that were separated by 5-minute breaks. The ISI was 9-20. The order of presentation of targets and standards was randomized with the restriction that no more than two targets were presented consecutively.

The participants received instructions on a computer screen about the fact that stimuli were going to be presented and that they had to learn when they had to press or to not press the button. Furthermore, after each trial, they received feedback by means of a counter. If they responded correctly, two points were added to the counter, whereas one point was subtracted if they responded incorrectly. The counter was initially set at 10 points and the number of points could not decrease below zero. The participants were instructed to earn as many points as possible. They had to respond to the target stimuli and were allowed to press the button with their preferred hand.

During the experiment, the participants sat comfortably in a chair in a sound-attenuating, dimly-lit cubicle (inside dimensions: 2 x 2.2 x 2 m). They were instructed to keep their eyes focused on the computer screen and to sit as still as possible.

## Data analysis

The data of two human participants were excluded from analysis because of excessive eye-blinking and that of one participant because she did not learn the task before the 40<sup>th</sup> target stimulus.

The EEG fragments within an epoch of 100 ms before onset and 1000 ms after onset were averaged, for each trial type separately, using the 100 ms prestimulus as baseline value. Separate averages were made for the first 20 target and standard stimuli (start of experiment) and for the last 20 target and standard stimuli (end of experiment), in order to be able to observe learning effects. All trials were included in the average, that is, both trials with correct and incorrect responses.

The recently proposed method of wavelet denoising (Quian Quiroga, 2000; Quian Quiroga & Garcia, 2003) was used in order to improve signal-to-noise ratio. The amplitudes of the human N100 and P300 components were defined as the maximum negative amplitude between 70 and 160 ms, and the most positive amplitude between 250 and 380 ms after stimulus onset, respectively.

Separate analyses of variance (ANOVA) were performed for trials at the start and at the end of the experiment in both species. Analyses were performed for both the N100 and P300 components, with Trial type (target and standard) and Electrode (Fz, Cz, Pz) as within subject factors. The Bonferroni correction was used for post-hoc tests and the level of significance was set at  $p < 0.05$  throughout.

## Results

### Behavioral responding

The participants had learned to press the button in response to the targets, but not to the standards, after a mean of 7.6 target trials (SD = 7.8).

### ERPs

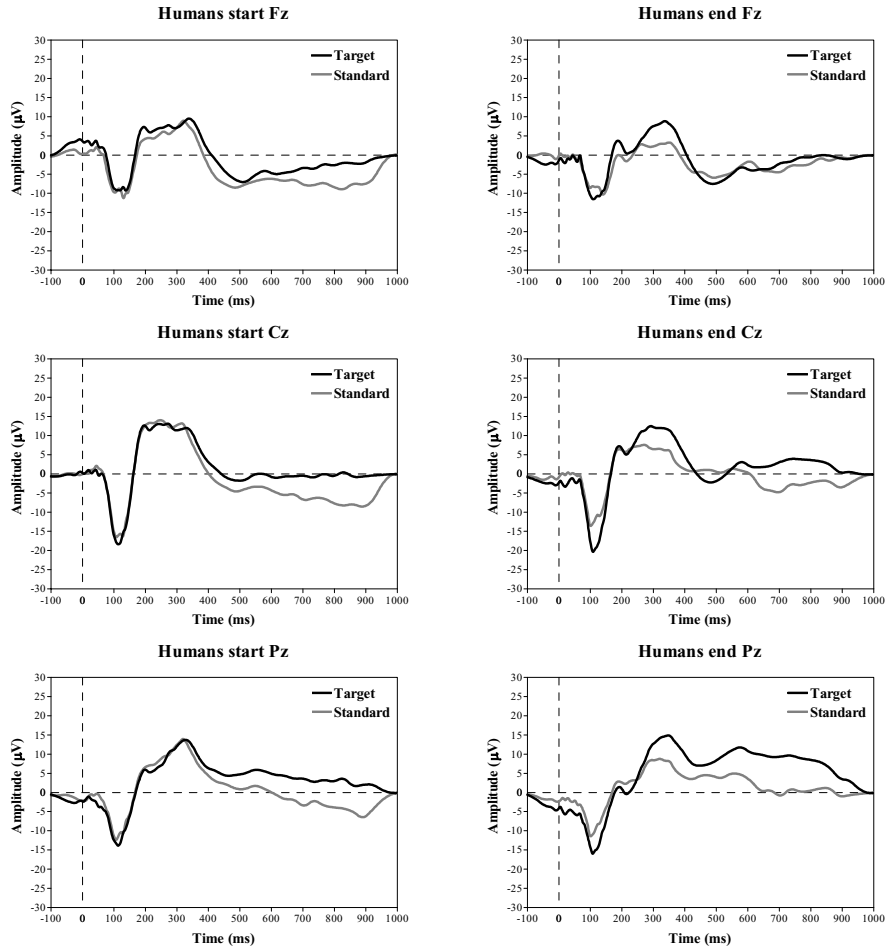
The ERPs at the start and end of the experiment are presented in Figure 1 for all three electrode positions. The N100 and P300 components are equally large in response to the target and standard stimuli at the start of the experiment. At the

end of the experiment, both components are larger at target than at standard stimuli.

### N100

A two-way ANOVA on the amplitudes of the N100 component at the start of the experiment revealed a main effect of Electrode,  $F(2, 13) = 25.74$ ,  $p < .001$ . The N100 was more negative at Cz than at Fz and Pz. No main effect of Trial Type ( $F = .12$ ) and no interaction ( $F = 1.29$ ) were found.

The two-way ANOVA on the N100 at the end of the experiment revealed main effects of Trial Type,  $F(1, 14) = 6.09$ ,  $p = .027$ , and Electrode,  $F(2, 13) = 12.74$ ,  $p$



*Figure 1.* Grand average ERPs in response to the target and standard stimuli at both the start and the end of the experiment, for the Fz, Cz, and Pz electrode positions. Note that the N100 and P300 were equally large in response to target and standard stimuli at the start of the experiment, whereas they were larger at targets compared to standards at the end.

= .001, but no interaction ( $F = 3.52$ ). The N100 was more negative at Cz than at Pz and it was more negative in response to the target than to the standard stimuli.

### *P300*

A two-way ANOVA on the amplitude of the P300 at the start of the experiment revealed a main effect of Electrode,  $F(2, 13) = 7.41$ ,  $p = .007$ . The P300 was larger at Cz than at Fz. No main effect of Trial Type ( $F = .35$ ) and no interaction ( $F = .49$ ) were found.

The ANOVA on the P300 at the end of the experiment showed a main effect of Trial Type,  $F(1, 14) = 5.59$ ,  $p = .033$ , but no effect of Electrode or an interaction effect. The amplitude was more positive in response to the target than to the standard stimuli.

## **Discussion**

The purpose of this experiment was to study the effects of learning on the human ERP. In an oddball task, the participants had to learn to respond to targets by pressing a button, but not to standards. The results showed that the N100 and P300 components were equally large at the start of the experiment, whereas the amplitudes of these components were larger in response to targets than to standards at the end of the experiment.

The results of the last phase of the experiment are in full accordance with the general oddball effects found in tasks with instruction (e.g., Katayama & Polich, 1996; Ochoa & Polich, 2000; Rockstroh et al., 1996; Sambeth et al., 2003). The finding that, under the present non-instruction condition, the N100 and P300 amplitude enlargements in response to target compared to standard stimuli needed time to develop, is novel.

Several researchers (Katayama & Polich, 1996; Polich, 1990a; Polich, Ellerson, & Cohen, 1996; Squires, Wickens, Squires, & Donchin, 1976) proposed that target probability affects the amplitude of the P300. However, the present results suggest that probability itself does not cause the differential P300 effects found in oddball tasks. Task relevance may at least be equally important, because the P300 only differed when the participants learned that the target was relevant, whereas the standard was not.

In conclusion, learning affects the N100 and P300 components of the human ERP. Targets only elicit larger amplitudes than do standards if the participants perform well on the task.

## **Chapter 6**

# **With long intervals, inter-stimulus interval is the critical determinant of human P300 amplitude**

A. Sambeth, J. H. R. Maes, and J. Brankačk

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### **Abstract**

Previous research, using short inter-stimulus intervals (1-4s), suggests that the P300 of the human event-related potential during oddball and single-stimulus tasks is mainly affected by target-to-target interval (TTI). The present study tested the validity of this claim at longer intervals. Participants were assigned to either an oddball task with an ISI of 9-20s or a single-stimulus task with an ISI of 9-20 or 40-90s. In the oddball task, the target elicited larger amplitudes than did the standard. When comparing the stimuli from the short- and long-ISI conditions with the target from the oddball condition, it was found that the P300 was more positive at long-ISI stimuli than at short-ISI stimuli or oddball targets, and short-ISI stimuli and oddball targets elicited equally large P300 amplitudes. These results suggest that, in oddball tasks with long intervals, besides cognitive factors, ISI rather than TTI crucially affects the P300 amplitude.

Descriptors: P300, oddball task, single-stimulus task, inter-stimulus interval (ISI), target-to-target interval (TTI)

Event-related potentials (ERP) are frequently studied in the so-called oddball task. In this task, frequently occurring standard stimuli are interspersed with infrequently occurring target stimuli and participants are asked to count the targets or to press a button in response to the targets. The most prominent ERP component elicited in the oddball task is the P300 component: a potential at 300 ms with a positive polarity. It is enlarged on target trials compared to standard trials (e.g., Fitzgerald & Picton, 1982; Katayama & Polich, 1996).

A further task that has frequently been used to elicit the P300 is the single-stimulus task, in which only relevant targets are presented to which the participants have to respond. This single-stimulus task appears to generate a P300 component of similar amplitude as is the case for the P300 elicited in the oddball task, suggesting that the same neural and cognitive mechanisms are engaged in the single-stimulus and oddball tasks (Polich & Margala, 1997; Polich, Eischen, & Collins, 1994).

Several factors may influence the amplitude of the P300 in response to targets. The first two factors mentioned below apply to stimuli in an oddball task; the third factor holds for both the oddball and single-stimulus paradigms. First, global stimulus probability, the mean probability during an experiment, has been shown to be negatively correlated with P300 amplitude (e.g., Katayama & Polich, 1996; Polich, 1990; Polich & Bondurant, 1997; Polich et al., 1994; Squires, Wickens, Squires, & Donchin, 1976). If the probability of a target is decreased, the amplitude of the P300 is increased. Second, the sequence length or local probability also affects the P300 amplitude in response to targets. Sequence length refers to the probability that a target will occur within a number of consecutively presented standards. The P300 in response to a target increases with increasing sequence length (Kilpeläinen et al., 1999; Polich & Bondurant, 1997; Squires et al., 1976). A third factor is the inter-stimulus interval (ISI). The P300 is more positive in response to long-ISI than to short-ISI conditions (Polich, 1990; Polich et al., 1994).

Global probability, local probability, and ISI have one thing in common. They all are related to the time between two consecutive targets (if the stimulus in a single-stimulus task is considered as a target because it requires a response). Therefore, it has been suggested that the changes in P300 amplitude are mainly due to the target-to-target interval (TTI) (Croft, Gonsalvez, Gabriel, & Barry, 2003; Gonsalvez et al., 1999), rather than to any of the other factors.

So far, the experiments that tried to determine the critical factors affecting the P300 amplitude always used relatively short ISIs of between 1 and 4 s (Croft et

al., 2003; Kilpeläinen et al., 1999; Polich & Margala, 1997). Whether the TTI hypothesis also holds for long ISI conditions is unknown. The present experiment was performed to answer this question. Participants either performed an oddball task with an ISI of 9-20 s, or a single-stimulus task with an ISI of 9-20 or 40-90 s. It was hypothesized that, if the TTI indeed is the determining factor of P300 amplitude, equally large P300 amplitudes should be elicited in response to the targets in the oddball and to the stimuli in the long-ISI single-stimulus conditions. Furthermore, the P300 in response to stimuli in the short-ISI single-stimulus condition should elicit smaller amplitudes than do the other two conditions.

## **Method**

### **Subjects**

Fifty-three students (eleven men, mean age 22 years) of the University of Nijmegen, The Netherlands, participated in the experiment. They were either paid for their participation, or received course credits. They were only allowed to take part in the study if they were healthy, did not use medication, and had no psychiatric history. Subjects who agreed to participate signed a written informed consent.

### **Electrode placement**

EEG activity was recorded using an electrode cap with tin leads from the Fz, Cz, and Pz sites according to the international 10-20 system, with the right mastoid as reference. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Impedance was less than 5 k $\Omega$  for all participants. EEG and EOG were filtered between 0.016 and 100 Hz and sampled at 512 Hz.

### **Procedure**

First, EEG and EOG electrodes were attached to the skin. Next, the participants were assigned to one of three conditions: short ISI, long ISI, or oddball task. Two auditory tones were used as stimuli: a 1000 Hz tone that served as target (all three conditions) and a 2000 Hz tone that served as a standard (only



oddball). Each stimulus had a duration of 50 ms, with 10 ms rise and fall times and an intensity of 70 dB.

Participants in the short-ISI condition were presented 60 target stimuli in one block with a random ISI ranging from 9 to 20 s. Participants in the long-ISI condition were presented 60 target stimuli, divided over three blocks that were separated by 5-minute breaks. The ISI was 40-90 s. The participants in the oddball condition were presented 60 target (probability 20%) and 240 standard stimuli, divided over three blocks that were separated by 5-minute breaks. The ISI was 9-20 (TTI of 40-90 s). The order of presentation of targets and standards in the oddball task was randomized with the restriction that no more than two targets were presented consecutively.

The present study was performed within the framework of a larger study on the effects of learning on the ERP. For this reason, participants were not instructed about the task requirements, but had to learn when to respond to stimuli. The participants received instructions on a computer screen about the fact that stimuli were going to be presented and that they had to learn when they had to press or not to press the button. Furthermore, after each trial, they received feedback by means of a counter, which was visible after each trial. If they responded correctly, two points were added to the counter, whereas one point was subtracted if they responded incorrectly. The counter was initially set at 10 points and the number of points could not decrease below zero. The participants were instructed to earn as many points as possible.

In all three conditions, the participants had to respond to the target stimuli. Thus, in the short and long ISI conditions, participants responded to all stimuli, whereas the participants in the oddball condition only responded to one of the two stimuli. The participants were allowed to press the button with their preferred hand. They were instructed to keep their eyes focused on the computer screen and to sit as still as possible.

### **Data analysis**

The data of eleven participants that showed excessive eye-blinking and/or that made too many errors (less than 40 correct target responses), were excluded from further analysis. The trials associated with incorrect responses, that is, no response to a target (all three conditions), or a response to the standard (only oddball), were excluded from analysis. The EEG fragments within an epoch of 100

ms before onset and 1000 ms after onset were averaged for all correct responses for each condition separately, using the 100 ms prestimulus as baseline value.

As the number of target trials in all conditions was relatively low, the recently proposed method of wavelet denoising (Quiñan Quiroga & Garcia, 2003) was used in order to improve signal-to-noise ratio.

The amplitude of the P300 component was defined as the maximum positive amplitude between 250 and 380 ms after stimulus onset.

First, an analysis of variance (ANOVA) on the P300 amplitudes in the oddball task was performed to confirm the usual oddball effects, with Trial type (target and standard) and Electrode position (Fz, Cz, Pz) as within subject factors. Next, an ANOVA was performed to compare the target P300 amplitude across conditions, with Electrode position (Fz, Cz, Pz) as within subject factor and Condition (oddball, short ISI, long ISI) as between subject factor. One-way ANOVAs with Bonferroni correction and simple main effect analyses were performed as post-hoc analyses. The level of significance was set at  $p < 0.05$  throughout.

## **Results**

Figure 1 shows the ERPs in response to the three conditions. The general oddball effect is present, as the P300 in response to targets was more positive than that to standards. Furthermore, it can be seen that, for all electrode positions, the P300 was larger in the long-ISI condition than in the other two conditions. The short-ISI condition elicited an equally large P300 amplitude as did the oddball condition.

The ANOVA using the data of the oddball condition revealed significant main effects of Trial type,  $F(1, 14) = 11.08$ ,  $p = .005$ , and Electrode,  $F(2, 13) = 9.18$ ,  $p = .003$ , but no interaction ( $F = .66$ ). The target elicited a larger P300 amplitude than did the standard and the P300 was more positive at Cz and Pz than at Fz.

The ANOVA using the P300 amplitudes from the targets in each condition revealed main effects of Condition,  $F(2, 39) = 9.63$ ,  $p < .001$ , and Electrode,  $F(2, 38) = 33.16$ ,  $p < .001$ , as well as a significant interaction between Condition and Electrode,  $F(4, 78) = 3.05$ ,  $p = .022$ . One-way ANOVAs for each electrode position showed significant Condition effects for each electrode,  $F(2, 39) = 3.32$ ,  $p = .046$ ,  $F(2, 39) = 7.88$ ,  $p = .001$ , and  $F(2, 39) = 15.52$ ,  $p < .001$ , for the Fz, Cz, and Pz site, respectively. At Cz and Pz, post-hoc comparison revealed that the P300 was more

positive in response to the targets in the long-ISI condition than in the other two conditions. At Fz, post-hoc analysis failed to reveal significant differences.

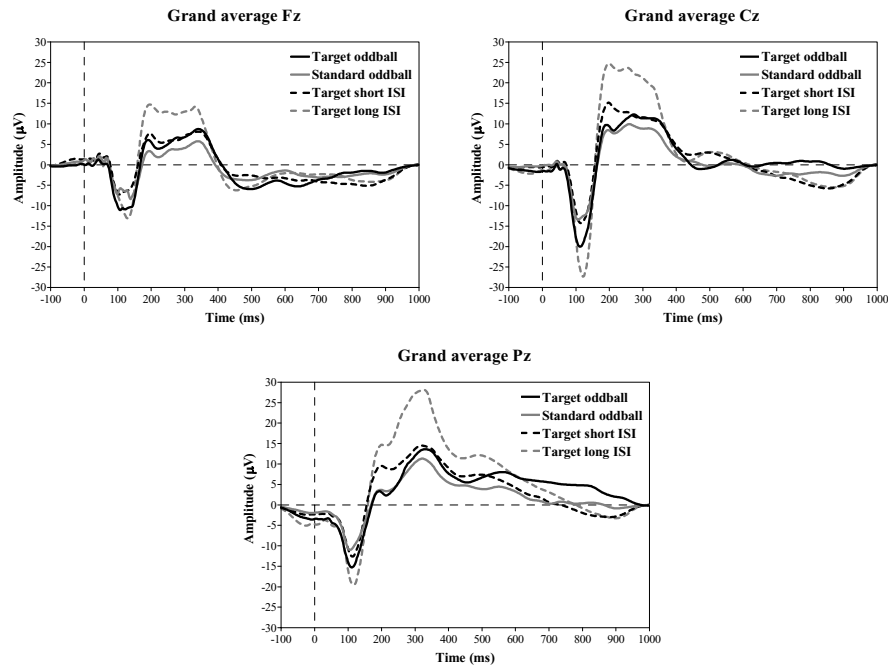


Figure 1. Grand average ERPs corresponding to each of the three conditions, oddball (targets and standards), short ISI (targets), and long ISI (targets), for each of the three electrode positions. Note the pronounced P300 amplitude difference between the long-ISI condition and the other two conditions.

## Discussion

Previous research (Croft et al., 2003; Gonsalvez & Polich, 2002; Gonsalvez et al., 1999), which only used relatively short inter-stimulus intervals (1-4 s), suggests that the P300 in response to oddball and single-stimulus tasks is mainly affected by target-to-target interval (TTI). The purpose of this study was to test the validity of this claim for longer ISI in a learning experiment. This was examined in an oddball condition (targets and standards) and two single-stimulus conditions (only targets) with varying ISIs that were longer than 9 s. Although the ISI was larger than usual in all conditions, the results of the oddball task revealed the common oddball effect: targets elicited a more positive P300 than did standards. Furthermore, the P300 was more positive in response to the targets in

the long-ISI condition than to the targets in the oddball condition for which an equivalent TTI was in effect. The P300 in the oddball and short-ISI conditions did not differ.

Our results imply that, contrary to the suggestions of the group of Gonsalvez (Croft et al., 2003; Gonsalvez & Polich, 2002; Gonsalvez et al., 1999), TTI is not the main determining factor of P300 amplitude. In the current study, the P300 was indeed larger in the long-ISI than in the short-ISI single-stimulus conditions, indicating evidence in favor of the TTI hypothesis. However, in the oddball condition with TTIs equivalent to ISIs used in the long-ISI condition, the P300 was smaller compared to the long-ISI condition.

A factor that might considerably affect the amplitude of the P300 is the ISI in general. This is because in the short-ISI condition, the P300 was less positive as in the long-ISI condition. Furthermore, the P300 was larger in the long-ISI than in the oddball condition, although the TTI was longer than 40 s in both conditions. This difference might be caused by the fact that standard stimuli were interspersed with the targets in the oddball condition, which led to a shorter ISI in this condition. A third argument is that with an ISI of 9-20 s, as was the case in the short-ISI and oddball conditions, the amplitude of the P300 was equally large. Apparently, the presence of 'odd' stimuli within a series of standards was not a requirement for obtaining a large P300 component, suggesting that ISI in itself was the determinant of the P300 amplitude.

Gonsalvez and Polich (2002) proposed that TTI is the critical determinant of P300 amplitude. They further demonstrated that the amplitude of the P300 increases with TTIs up to 6-8 s and is relatively stable thereafter, suggesting a ceiling effect. In this respect, it has been suggested that P300 amplitude fluctuations may be due to a recovery cycle (Fitzgerald & Picton, 1981; Gonsalvez & Polich, 2002). Compared to long intervals, the P300 will be small with short intervals, because the system requires time to recover from the very recent P300 production. The fact that in our experiment the P300 in response to targets was more positive in the long-ISI condition than in the short-ISI condition suggests that the P300 amplitude does not reach its maximum with TTIs of 6-8 s, as was suggested by Gonsalvez & Polich (2002). If a ceiling effect exists, it will be found at much longer intervals. As we did not use intervals longer than 40-90 s in the present study, we can only suggest that the P300 amplitude increases up to at least 40-90 s, and possibly even thereafter.

An issue deserving further consideration is the instruction given to the participants in the experiment. Usually, participants are instructed about the

task requirements. In this experiment, however, the participants had to learn by trial-and-error when they had to press the button. It is possible that the participants, at least those in the single-stimulus conditions, stayed more alert than is the case in tasks usually employed, because they might have thought that there was more to the task than responding to one stimulus and that the task would change during the experiment. Furthermore, as they had to earn as many points as possible, they were extra motivated during the task. Therefore, the P300 amplitudes in the long-ISI condition may have been larger compared to P300 amplitudes usually obtained in oddball and single-stimulus tasks (Fitzgerald & Picton, 1981; Gonsalvez et al., 1999; Polich & Bondurant, 1997).

Another possible explanation for the larger P300 in the long-ISI than in the oddball condition is that the cognitive load was larger in the oddball task than in the single stimulus tasks. This may have led to less positive P300 amplitudes in the oddball paradigm compared to the long-ISI paradigm and to the relatively large P300 response at standards. A decreased P300 amplitude in response to targets was also found in an oddball task in a dual task study with a high cognitive load (Singhal, Doerfling, & Fowler, 2002). However, the P300 in that study was absent in response to the standards, which is not in line with a differential cognitive load explanation of our results. Nevertheless, we cannot exclude the possibility that effects of cognitive load and ISI interact.

In conclusion, this study revealed that, in a learning experiment with long intervals, cognitive factors together with the inter-stimulus interval, rather than TTI itself, affect the P300 amplitude in an oddball task.

## **Chapter 7**

# **On the electrophysiological correlates of occasion setting as observed in feature discrimination tasks**

A. Sambeth, J. H. R. Maes and A. M. L. Coenen

*Submitted in revised form*

### **Abstract**

Occasion setting refers to the ability of a feature stimulus to signal or retrieve the association between a target stimulus and some other stimulus. The purpose of this study was to assess the electrophysiological (ERP) correlates of occasion setting in humans, using a procedure that is known to induce occasion setting in animals. Furthermore, we studied whether occasion setting actually is present during these tasks. Experiment 1 involved the requirement to respond to an auditory target stimulus (A) if it followed one visual feature (X), but to not respond if it followed another visual feature (Y) ( $X \rightarrow A+ / Y \rightarrow A-$ ). In Experiment 2, participants learned to respond to Tone 1 (A) if it followed a feature (X) and to Tone 2 (B) if it was presented alone, and to not respond if Tone 1 was presented alone and if Tone 2 followed the feature ( $X \rightarrow A+ / A- / B+ / X \rightarrow B-$ ). The participants' behavioral performance was consistent with the notion of occasion setting and the Readiness Potential was more negative on '+' than on '-' trials. However, the amplitudes of the P300 and N200 components elicited by the target(s) did not differ on these trial types, although, in Experiment 1, the feature's P300 was marginally higher on '+' than on '-' trials. These data suggest the simultaneous, independent operation of occasion-setting and simple associative processes and the absence of a clear ERP correlate of occasion setting on components earlier than 400ms.

Descriptors: direct association, event-related potential, N200, occasion setting, P300, Readiness Potential

In a Pavlovian feature-positive discrimination procedure, a target stimulus (conditioned stimulus, CS) is followed by a biologically relevant event (unconditioned stimulus, US) if it is preceded by a feature stimulus, and it is not followed by the US if presented alone. Previous research (for reviews see Holland, 1992; Schmajuk & Holland, 1998) has shown that an association is established between the target stimulus and the US, and that this association is modulated by the feature stimulus. In other words, the feature sets the occasion for the presence of a CS-US link. It is, however, possible that, together with its modulatory capacity, the feature may establish a direct association with the US. This latter association is not primarily responsible for the behavior in the feature-positive procedure, but may exist independently from occasion setting (Holland, 1992).

In order to conclusively know that the feature modulates the CS-US associations, rather than that performance is simply based on direct feature-US associations, control manipulations like counter-conditioning or extinction can be implemented on the feature stimulus (for a review see Holland, 1992). In these cases, the associative value of the feature is explicitly changed. If the feature primarily had modulatory powers, the explicit change should have no direct effect on discrimination performance at feature-target presentations. If it had only simple associative functions, however, responding to the feature-target presentations would be changed (for a review see Schmajuk & Holland, 1998). Another strategy is to assess the potential of the feature to modulate responding to another target (transfer test). If the feature functions as occasion setter, transfer should be selective to similarly trained targets, whereas it should transfer to any target in the case of direct associations.

Occasion setting has extensively been studied in animals in both Pavlovian (for reviews see Holland, 1992; Schmajuk & Holland, 1998) and operant conditioning paradigms (Holland, 1991, 1995a, 1995b, 1997; Holland, Parsons, & Hamlin, 1997). The difference between these paradigms is, that, in the operant case, the feature modulates a discriminative stimulus-response-US association, rather than a CS-US association. In the present study, an operant occasion-setting paradigm was used.

The animal studies have shown that there are conditions that favor the occurrence of occasion setting rather than simple conditioning. Holland (1989) showed that the likelihood of occasion setting is larger if the feature and target cues have a different modality than if both stimuli are of the same modality. Furthermore, temporal factors play a role. If the termination of the feature

stimulus precedes the onset of the target stimulus (serial presentation), occasion setting is more likely than if the two stimuli are presented simultaneously (e.g., Holland, 1986, 1989; Ross & Holland, 1981). Finally, the likelihood of occasion setting is larger if the intertrial intervals are relatively large (Holland, 1995a, 1997).

Occasion setting has extensively been studied in animals; a smaller number of experiments has been performed in humans (Baeyens, Crombez, De Houwer, & Eelen, 1996; Baeyens, Hendrickx, Crombez, & Hermans, 1998; Baeyens, Vansteenwegen, Hermans, Vervliet, & Eelen, 2001; Dibbets, Maes, & Vossen, 2002; Dibbets, Maes, Van den Berg, De Wit, & Vossen, 2002; Hardwick & Lipp, 2000; Young, Johnson, & Wasserman, 2000). Baeyens and co-workers (1996, 1998) did not find occasion setting in a serial flavour-flavour conditioning task. The remaining five studies, on the other hand, did find support for occasion setting. Hardwick and Lipp (2000) reported differential responding to targets in a serial feature-positive task in a first experiment, but concluded on the basis of a second experiment that responding in their experiments was probably due to two different types of association, a simple association between the feature and the US and occasion setting by that same feature. However, they did not use any control procedures in their experiments. The other four articles (Baeyens et al., 2001; Dibbets, Maes, & Vossen, 2002; Dibbets, Maes, Van den Berg et al., 2002; Young et al., 2000) did control for the contribution of simple associations by performing extinction, transfer, or counter-conditioning manipulations on the feature stimulus in simultaneous and serial feature-positive tasks and showed that the performance at feature-target presentations remained intact after at least the extinction and counter-conditioning manipulations in the serial condition. They, therefore, concluded that the feature had obtained occasion-setting powers.

As reported in the previous paragraph, Baeyens and colleagues (1996, 1998) were not able to find occasion setting in a feature-positive discrimination task. Using rats as subjects, Bouton and Nelson (1998) also failed to find evidence for occasion setting in both simultaneous and serial tasks. They only found evidence for direct feature-US associations and proposed that there are many ways to reach a single behavioral result (reliable discrimination performance), which may cause different researchers to obtain different results. In this respect, another explanation comes from Pearce (1987, 1994). Instead of establishing target-US links, which are modulated by a feature, the feature and the target may be seen



as one compound stimulus, and responding depends on the associative strength of the compound and the US.

In sum, results of the human studies have shown that occasion setting can occur in humans in feature-positive discrimination tasks. As in animal studies, the temporal order between the feature and the target, the interstimulus interval, proved to be an important factor (Baeyens et al., 2001; Dibbets, Maes, & Vossen, 2002; Dibbets, Maes, Van den Berg et al., 2002; Young et al., 2000). In each of these studies, occasion setting did occur with serial stimulus presentations, whereas it did not with simultaneous presentations. Furthermore, most of the studies presenting at least partial evidence for occasion setting (Baeyens et al., 2001; Dibbets et al., 2002; Hardwick & Lipp, 2000) used different modalities for feature and target stimuli, whereas Baeyens et al. (1996, 1998) did not. It, therefore, seems that in both animal and human studies, the use of different modalities for features and targets is an important factor in obtaining evidence for occasion setting. Hence, to promote occasion setting in the present study, we used visual feature and auditory target cues in serial feature discrimination tasks.

Event Related Potentials (ERP) are averaged electroencephalographical (EEG) potentials triggered by, and time-locked to, sensory stimuli (Näätänen, 1990). One of the ERP components that has extensively been studied is the P300 component. It is associated with stimulus evaluation and expectancy (Donchin, 1981), and is larger in amplitude if a stimulus is relevant, such as indicating a need to press a button, than if it is of no importance (e.g., Garcia-Larrea, Lukaszewicz, & Mauguiere, 1992; Sambeth et al., 2003). Further components frequently studied are the N200 component, which is suggested to be a non-motor inhibition process and which is more negative if a subject has to refrain from responding than when making a response (Falkenstein, Hoormann, & Hohnsbein, 1999; Bokura, Yamaguchi, & Kobayashi, 2001), and the Readiness Potential (RP), which is reflected by a sustained negativity before a voluntary movement and which can be referred to as attention selectively to the output system (Brunia, 1993).

To our knowledge, ERP studies have not been performed so far using feature discrimination procedures. However, tasks similar to feature discrimination tasks have investigated the N200 and P300 components of the ERP. Using the continuous performance test (CPT), in which participants have to respond to a letter (say X) when it is preceded by another letter (say A) and to withhold responding when another letter follows the A, it has been shown that the amplitude of the P300 component to X is larger than that to other letters at

parietal electrode sites (Bokura et al., 2001; Tekok-Kilic, Shucard, & Shucard, 2001). Furthermore, the N200 component is less negative at the letter X than at the other letters mainly at frontal leads (Bokura et al., 2001).

Other tasks that resemble feature discrimination procedures are Go/NoGo and oddball tasks. In both tasks, participants are instructed to respond to one, infrequent stimulus (target stimulus), but to withhold responding to another, frequent stimulus (standard stimulus). It has been shown that the P300 has a larger amplitude following target stimuli than after standard stimuli, again at parietal leads (e.g., Barrett, Neshige, & Shibasaki, 1987; Ochoa & Polich, 2000, Rockstroh et al., 1996; Sambeth et al., 2003). The N200 component shows more negativity in response to the NoGo stimuli than to the Go stimuli (Falkenstein et al., 1999).

The CPT tasks and Go/NoGo tasks, however, importantly differ from feature discrimination procedures. For example, none of these procedures involves a 'target' stimulus that has an ambiguous meaning in that it requires different responses on different associations. This also holds for the task most similar to the feature discrimination procedures, the CPT task, because here, the target X (which is consistently presented after 'feature' A) must always be responded to. Moreover, in CPT or Go/NoGo paradigms, the participants receive task instructions, whereas subjects have to learn the discrimination by trial-and-error in a feature discrimination procedure. The CPT task differs on another important aspect. Distracting non-relevant stimuli are interspersed with the relevant stimuli in this task (Fallgatter and Strik, 1999; Bokura et al., 2001), whereas all stimuli are relevant in the feature discrimination procedures. None of these studies have investigated the RP, as participants have to exert immediate responses. In the present study, the response of the participants was delayed, which made it possible to investigate this component.

As the CPT and Go/NoGo tasks differ substantially from feature discrimination procedures it is not clear to what extent P300, N200, and RP components are affected in these procedures. Therefore, we investigated the possible involvement of these components in occasion setting. As not all behavioral studies have been able to find evidence for occasion setting in feature discrimination procedures (e.g., Bouton & Nelson, 1998), we further attempted to assess whether responding indeed depended on occasion setting, or rather on simple associations.

**Experiment 1**

In the first experiment, we used a procedure with elements that may induce occasion setting. Participants had to learn to press a button in response to a target A if it followed a feature X. If A was preceded by a feature Y, they did not have to make any response. Next, we tested whether discrimination performance was based on occasion setting or simple conditioning. We used a counter-conditioning procedure on the feature stimulus implying that participants learned to respond to single presentations of Y, and to not respond to single presentations of X. In the final test phase, features X and Y were again followed by target A. We assessed whether participants responded in the same way as in the first, or the second phase. If X and Y primarily functioned as occasion setters participants should, in the test phase, press a button at A after the presentation of X, but not after Y. If X and Y functioned as simple conditioned stimuli and discrimination performance was primarily based on this function, responses should be generated in response to Y, or in response to A when it was preceded by Y, and not when preceded by X.

Furthermore, we studied whether the P300, N200, and RP components differed between X and Y presentations (visual ERP, henceforth called VEP), and between A following X and A following Y (auditory ERPs, henceforth called AEP). The following hypotheses might be generated with regard to these ERPs.

If X and Y primarily functioned as occasion setters, no differences should be found at the P300 and N200 components during the features in the first phase, because both stimuli have the same meaning in that the participants do not have to respond directly to any of them. As the features only have modulatory properties instead of direct associative properties, they should be processed in similar ways. The targets, having associative properties in case of occasion setting, should, however, be differentially processed, which might be reflected in differences at the P300 and N200 components in response to A after X versus A after Y. Furthermore, it is expected that participants show a RP at stimuli to which they make a response and not at the other stimuli. The same effects on the three ERP components should be present in the final test phase. In the second phase, in which only direct associations can be formed, the P300 and N200 components of the features X and Y should be differentially affected.

If X and Y merely functioned as simple associative stimuli, differences are expected at the P300 and N200 components following X and Y in all three phases.

No differences are anticipated at the P300, N200 or RP, however, at A after X versus A after Y.

## Materials and Methods

### *Participants*

Forty-four students (four men, mean age 23 years) of the University of Nijmegen, The Netherlands, participated in the experiment. They were either paid for their participation, or received course credits. They were only allowed to take part in the study if they were healthy, did not use medication, and had no psychiatric history. Participants who agreed to participate signed a written informed consent.

### *Apparatus*

The participants were tested in a sound-attenuating, dimly-lit cubicle (inside dimensions: 2 x 2.2 x 2 m). The participants were seated in a comfortable chair. A 17" computer monitor, which was used to present visual and auditory stimuli, was placed 1.5 m in front of the participant. A black and a grey circle with a radius of 6 cm, which were presented in the middle of the screen on a white background, were used as visual stimuli. A 1500-Hz, 70 dB(A) tone served as auditory stimulus. Participants had a button in their right hand during the experiment. Registrations of EEG, button presses, and presentation of the visual and auditory stimuli were recorded and controlled by a standard personal computer.

### *Electrode placement*

EEG activity was recorded using an electrode cap with tin leads from the Fz (frontal), Cz (central), and Pz (parietal) sites according to the international 10-20 system, with the right mastoid as reference. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Horizontal EOG recordings were made from the outer canthus of the right eye; vertical EOG recordings were made from an electrode placed supra orbital to the right eye. Impedance was less than 5 k $\Omega$  for all participants. EEG and EOG were filtered between 0.016 and 100 Hz and sampled at 512 Hz.

### *Procedure*

First, EEG and EOG electrodes were attached to the skin. Next, participants took part in a three-phase serial feature-discrimination task. The visual stimuli were used as features (X and Y); the auditory stimulus served as target (A). For half the participants, the black circle was used as feature X and the grey as Y,

whereas for the other half the grey circle was X and the black one Y. All visual and auditory stimuli had a duration of 1 s. The time between the end of the feature and the onset of the target was 5 s (interstimulus interval; ISI). The interval between the trials was random between 10 and 14 s (intertrial interval; ITI).

In Phase 1, half the trials consisted of feature X followed by target A ( $X \rightarrow A$ ), whereas the other half consisted of Y followed by A ( $Y \rightarrow A$ ). The participants had to learn to press a button upon termination of A, but only when it was preceded by X ( $X \rightarrow A+$ / $Y \rightarrow A-$ ). After each trial, they received feedback on the computer screen about their task performance (correct, incorrect, or too fast). Thirty-five trials of each type were presented in this phase in a semi-random order, with no more than three trials of the same type in a row. The participants received a 5-minute break after the first phase.

In Phase 2, the participants received 5 presentations of each of the two trial types of Phase 1. Next, the counter-conditioning phase started. X and Y were now presented alone, and participants had to learn to press the button after the presentation of Y (Y+), but not after X (X-). Thirty trials of each type were presented in a semi-random order, with no more than three trials of each type in a row. The participants again received feedback on their performance.

Phase 3 immediately followed Phase 2. As in Phase 1, presentations of the features X and Y were followed by target A, with the exception that no feedback was presented after the trials. Ten trials of each type were presented, with no more than three trials of the same type within a row. Half the participants first received the  $X \rightarrow A$  trial, whereas the other half were first presented  $Y \rightarrow A$ .

The participants only received instructions before the experiment about the fact that stimuli were going to be presented and that they had to learn when they had to press or to not press the button. The participants were allowed to press the button with their preferred hand, and were further told not to press the button during the presentation of a stimulus and to wait until the stimulus had ended. The experiment was conducted in the afternoon.

The participants sat comfortably in their chair during the experiment and were instructed to keep their eyes focused on the computer screen. They were instructed to sit as still as possible.

#### *Data analysis*

Behavioral analysis. The criterion for Phase 1 to be considered as learned was that correct responses were made to at least two  $X \rightarrow A+$  trials and to at least two  $Y \rightarrow A-$  trials in a row, whereas the criterion in Phase 2 was that correct responses

were made to at least one X- and one Y+ trial successively. In Phase 3, the participants did not receive any feedback. For each trial in Phase 3 it was determined if participants responded in the same way as in Phase 1, that is, if they did make a response to A on X→A, and not on Y→A trials. Responses according to Phase 1 were scored as 1; other responses were scored as 0. Mean scores across participants were then calculated for each trial. In order to examine whether the participants significantly responded as in Phase 1, a Chi-square test was employed for each of the 20 trials in this phase.

**EEG analysis.** The EEG was visually checked off-line for EOG activity and other artifacts. ERPs generated by stimuli that were presented in the presence of artifacts were excluded from further analysis. Furthermore, trials on which incorrect responses were made were also excluded from analysis. Incorrect responses in Phase 1 were X→A+ trials on which no response was made after A, and Y→A- trials on which a response was made. In Phase 2, responding to X- and not responding to Y+ were considered incorrect.

ERPs were calculated in response to the features (VEP) and the targets (AEP and RP). Separate averages for each trial type (X→A+, Y→A-, X-, Y+, test X→A, test Y→A) were determined for each individual. Grand averages were constructed for each trial type. The EEG fragments within an epoch of 100 ms before stimulus onset and 1000 ms after stimulus onset were averaged for all corresponding trials. The mean amplitude of the 100 ms before stimulus onset was used as a baseline value.

The ERP components were determined on the basis of the individual and grand average ERPs. Table 1 summarizes the components in response to both the features and the targets that were determined for statistical analysis.

Due to technical problems, the data from two participants were excluded from analysis.

*Table 1.* ERP components of the features and targets of interest with their corresponding latency ranges after stimulus onset

	Features	Targets
N200	240-350 ms *	210-300 ms *
P300	280-400 ms *	250-380 ms *
Readiness Potential	---	900-1000 ms **

\* Maximum amplitude within window

\*\* Mean amplitude within window

Statistical analysis of the EEG data. Multivariate analyses of variance (MANOVAs) with Trial type (X and Y for VEP, A+ and A- for AEP; 2 levels), Phase (conditioning, counter-conditioning, and test; 3 levels for VEP, conditioning and test; 2 levels for AEP), and Electrode position (Fz, Cz, and Pz) as within-subject factors were performed for the amplitudes of each of the components. The Bonferroni correction was used for post-hoc tests. The level of significance was set at 0.05 throughout.

## Results & Discussion

### *Behavioral analysis*

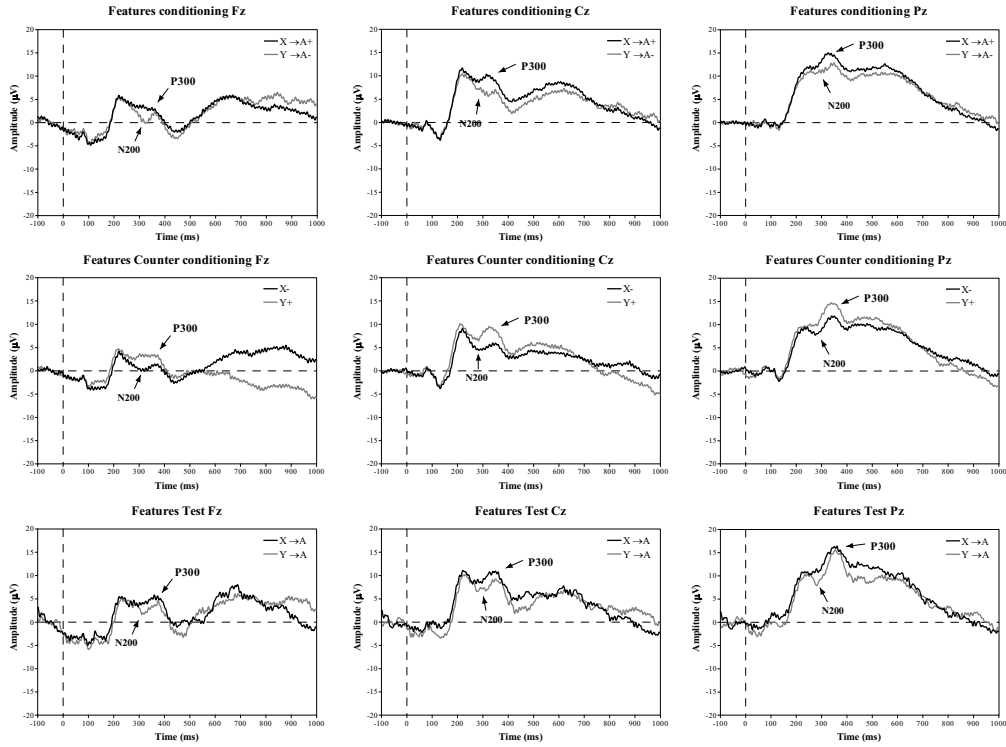
Four participants did not fulfill the learning criterion used in Phase 1. Their data were, therefore, excluded from further analysis. All other participants accomplished Phases 1 and 2. The mean score of both Trial 1 and Trial 2 of the Test phase, Phase 3, was 0.66, which just failed to be above chance level,  $\chi^2=3.79$ ,  $p=.052$ . The scores of all other trials were at least 0.80, and this was significantly different from chance level,  $\chi^2\geq 15.12$ ,  $p<.001$ . The participants, thus, did not respond in the same way as in Phase 1 during Trial 1 and Trial 2, whereas they did at all other trials.

The reason why participants did not respond in the same way during the first trials of Phase 3 as they did during Phase 1 may be a surprise effect of the sudden reappearance of targets. Even without receiving feedback, the participants may be said to have 're-learned' the task within the first two trials in the sense that they learned to again expect a target after each feature, and from Trial 3, participants started responding as in Phase 1. The fact that Phase 1 responses did recover from Trial 3 on may suggest that the participants used an occasion-setting strategy in this experiment.

### *ERP overview*

Figure 1 shows the VEPs in response to the features in the conditioning, counter-conditioning, and test phases (Phases 1, 2, and 3, respectively) for the Fz, Cz, and Pz electrode sites. The mean amplitudes of the P300 and N200 in response to the features are shown in Table 2. A large P300 is present at Cz and Pz, whereas an N200 is present at all electrodes (see Figure 1). The amplitudes of the P300 are larger after presentations of X than of Y in Phases 1 and 3, whereas the amplitudes are larger after presentations of Y compared to X in the counter-conditioning phase. The N200, on the other hand, seems to be more negative in all

phases at trials on which no response had to be made than at the trials requiring a response. No clear RP can be seen during Phases 1 and 3. Therefore, this component was not analyzed for the features. Figure 2 shows the AEPs in response to the targets in the conditioning and test phases (Phases 1 and 3, respectively) and the mean amplitudes of the P300, N200, and RP components in response to the targets can be found in Table 2. A P300 can be seen at Pz, an N200 at Fz, and a clear RP at Fz and Cz. No profound trial type effects can be seen for the P300 component. The N200 seemed more negative in response to A if preceded by Y than if preceded by X. Furthermore, the RP is more negative in response to A if it followed X than if it followed Y.



*Figure 1.* Grand average ERPs in response to the feature stimuli in Experiment 1. The upper three graphs show the Fz, Cz, and Pz sites in response to  $X \rightarrow A^+$  (black lines) and  $Y \rightarrow A^-$  (grey lines) trials during the conditioning phase (Phase 1). The middle three graphs illustrate the Fz, Cz, and Pz VEPs of  $X^-$  (black) and  $Y^+$  (grey) trials during the counter-conditioning phase (Phase 2). The bottom three graphs show Fz, Cz, and Pz sites in response to  $X \rightarrow A$  (black) and  $Y \rightarrow A$  (grey) trials during the test phase (Phase 3).



*VEP*

A MANOVA on the amplitudes of the P300 component showed several significant effects. A significant interaction was found between Trial type, Phase, and Electrode,  $F(4, 34) = 2.66, p = .049$ . Additionally, an interaction between Trial type and Phase was present,  $F(2, 36) = 8.76, p = .001$ . Finally, a main effect of Electrode,  $F(2, 36) = 68.69, p < .001$ , and Phase,  $F(2, 36) = 14.56, p < .001$ , were found. Post-hoc analyses showed that, for each Trial type and Phase, the amplitudes of the P300 component were more positive at Pz than at Cz and Fz, and amplitudes were more positive at Cz compared to Fz. Additionally, the amplitudes of the P300 were more positive in Phase 3 than in Phase 1. Post-hoc analysis of the Trial type x Phase interaction showed the following effects. Amplitudes were marginally larger in response to X→A+ than to Y→A- trials in Phase 1,  $F(1, 37) = 3.30, p = .077$ . In Phase 2, the amplitude of Y+ trials was more positive than that of X- trials,  $F(1, 37) = 12.49, p = .001$ . Phase 3 showed marginally significant effects,  $F(1, 37) = 3.29, p = .079$ . The amplitudes were slightly more positive in response to X→A than to Y→A stimuli.

The MANOVA on the amplitudes of the N200 component showed an interaction between Trial type and Phase,  $F(2,36) = 8.49, p = .001$ , and a main effect of Electrode,  $F(2,36) = 48.44, p < .001$ . Post-hoc analyses of the Electrode factor showed that the N200 component was more negative at Fz than at Cz and Pz, and amplitudes were more negative at Cz compared to Pz. Post-hoc analysis of the Trial type x Phase interaction showed the following effects. The amplitude of the N200 did not significantly differ between Trial types in Phases 1 and 3. In Phase 2, however, there was an effect. For each of the electrode sites, the N200 was more negative in response to X- than in response to Y+.

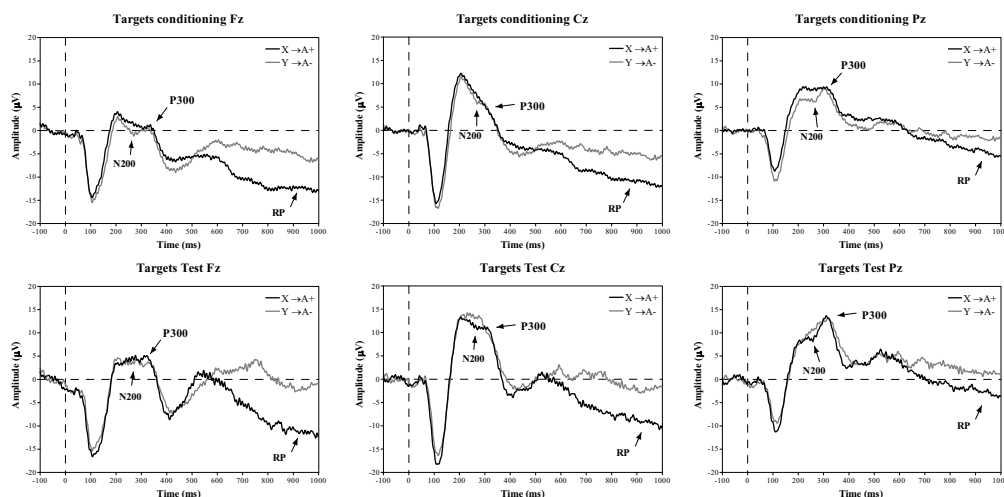
The results on the amplitudes of the VEP P300 and N200 components show that the counter-conditioning phase did have an effect. The amplitudes in response to X and Y reversed in Phase 2 compared to Phase 1. During the last phase, the amplitudes stabilized back to the level of Phase 1. With regard to the P300 component, the responses to X were again slightly larger than responses to Y in Phase 3. These effects may be a result of a simple conditioning effect of the feature stimulus, and thus provide no evidence for occasion setting.

*AEP*

A MANOVA on the amplitudes of the P300 component showed a significant interaction between Phase and Electrode,  $F(2, 36) = 6.14, p = .005$ . It also revealed a main effect of Phase,  $F(1, 37) = 33.32, p < .001$  and of Electrode,  $F(2, 36) = 33.54, p < .001$ . The amplitude of the P300 was larger in Phase 3 than in

Phase 1 ( $p < .05$ ). Furthermore, the amplitudes of the P300 were more positive at Cz and Pz compared to Fz.

A MANOVA on the amplitudes of the N200 component revealed interactions between Phase and Electrode,  $F(2, 36) = 10.88$ ,  $p < .001$ , and between Trial type and Electrode,  $F(2, 36) = 5.34$ ,  $p = .009$ . Furthermore, a main effect of Electrode was found  $F(2, 36) = 40.31$ ,  $p < .001$ . Post-hoc analyses of the Electrode factor showed that the N200 component amplitude was more negative at Fz compared to Cz and Pz. The analyses of the interactions showed that, only at Pz in Phase 1, the amplitudes at  $Y \rightarrow A^-$  trials were more negative than those at the  $X \rightarrow A^+$  trials.



*Figure 2.* Grand average ERPs in response to the target stimuli in Experiment 1. The upper three graphs show the Fz, Cz, and Pz sites in response to  $X \rightarrow A^+$  (black lines) and  $Y \rightarrow A^-$  (grey lines) during the conditioning phase (Phase 1). The bottom three graphs illustrate the Fz, Cz, and Pz AEPs in response to  $X \rightarrow A$  (black) and  $Y \rightarrow A$  (grey) trials of the test phase (Phase 3).

In the introduction, we hypothesized that if participants use an occasion-setting strategy, significant differences should be found regarding the amplitudes of the P300 and/or N200 components during Phases 1 and 3. In Phase 1, the N200 component did differ between  $X \rightarrow A^+$  and  $Y \rightarrow A^-$  presentations, but only at Pz. As the N200 component is usually most prominent at frontal leads (Falkenstein et al., 1999; Bokura et al., 2001), it is not likely that this result reflects the response inhibition at  $Y \rightarrow A^-$  trials which would be indicative of occasion setting. Furthermore, no effects were found for the P300. Therefore, the results of the targets can be explained best by simple conditioning effects.

Table 2. Mean amplitudes (in microvolts) and standard deviations (in parenthesis) of the P300, N200, and RP ERP components of the features (Experiment 1) and targets (Experiment 1 and 2), for each electrode site

Experiment	Modality	Phase	Trial type	P300			N200			RP		
				Fz	Cz	Pz	Fz	Cz	Pz	Fz	Cz	Pz
Exp. 1	Features (VEP)	Phase 1	X→A+	7.57 (8.36)	13.67 (8.90)	18.29 (8.47)	-1.13 (8.56)	3.45 (7.68)	7.21 (6.75)	-	-	-
				6.52 (7.62)	11.68 (6.69)	16.68 (6.60)	-3.26 (8.27)	1.74 (6.36)	6.14 (5.63)	-	-	-
			Y→A-									
		Phase 2	X-	5.73 (8.05)	9.62 (7.79)	15.19 (7.33)	-3.68 (7.74)	-21 (7.36)	4.19 (6.46)	-	-	-
				7.22 (7.14)	12.80 (7.16)	17.49 (6.84)	-1.19 (7.44)	2.63 (6.67)	5.65 (6.58)	-	-	-
			Y+									
	Targets (AEP)	Phase 3	X→A	12.04 (11.71)	17.40 (11.23)	21.99 (10.44)	-2.68 (10.96)	1.90 (9.62)	5.16 (7.84)	-	-	-
				10.16 (11.54)	14.78 (8.72)	19.59 (7.07)	-3.19 (11.12)	.87 (8.08)	3.25 (6.57)	-	-	-
			Y→A									
		Phase 1	X→A+	5.95 (6.24)	10.76 (7.00)	12.59 (5.91)	-3.61 (5.88)	2.44 (6.71)	4.52 (5.03)	-12.35 (15.58)	-11.91 (12.32)	-6.13 (8.36)
				5.41	10.75	11.64	-4.89	1.80	2.14	-6.02	-5.45	-2.50
			Y→A-	(6.61)	(5.66)	(5.19)	(6.49)	(5.54)	(4.47)	(9.60)	(5.97)	(6.60)

Table 2 (continued) . Mean amplitudes and standard deviations of the P300, N200, and RP ERP components of the features (Experiment 1) and targets (Experiment 1 and 2), for each electrode site

Experi- ment	Modality	Phase	Trial type	P300			N200			RP		
				Fz	Cz	Pz	Fz	Cz	Pz	Fz	Cz	Pz
Exp. 1	Targets (AEP)	Phase 3	X→A	11.27 (11.07)	17.83 (11.21)	17.72 (10.15)	-4.06 (10.22)	3.20 (9.42)	2.44 (7.39)	-11.22 (19.20)	-9.26 (13.14)	-2.92 (10.47)
				11.04 (10.85)	18.96 (10.18)	18.43 (9.07)	-3.48 (10.07)	4.81 (8.30)	2.62 (7.08)	-1.78 (16.05)	-2.24 (12.78)	1.31 (9.28)
			Y→A	6.71 (5.59)	11.14 (6.12)	13.89 (5.99)	-1.23 (4.48)	3.37 (5.04)	3.96 (4.45)	-7.37 (8.33)	-7.33 (6.13)	-2.89 (5.28)
				5.58 (4.96)	9.97 (6.78)	12.54 (6.38)	-3.35 (4.37)	2.02 (5.32)	2.82 (4.69)	-3.95 (9.65)	-5.13 (6.25)	-2.61 (4.42)
Exp. 2	Targets (AEP)		B+	5.96 (6.93)	9.58 (6.49)	12.21 (5.09)	-2.62 (5.98)	2.09 (5.34)	2.04 (5.35)	-10.64 (8.38)	-10.17 (5.56)	-5.43 (4.49)
				5.36 (6.66)	9.53 (7.02)	12.82 (5.54)	-3.00 (5.08)	2.04 (5.41)	3.09 (4.59)	-1.41 (8.46)	-2.90 (5.04)	-68 (3.97)
		X→B-										

*RP*

A MANOVA on the mean amplitudes of the RP showed an interaction between Trial type and Electrode,  $F(2, 36) = 6.44$ ,  $p = .004$ . Post-hoc analyses per Trial type showed that Fz and Cz amplitudes were more negative than those of Pz at each Trial type. The MANOVA further showed a main effect of Trial type,  $F(1, 37) = 9.78$ ,  $p = .003$ . The mean amplitude on  $X \rightarrow A+$  trials was more negative than that on  $Y \rightarrow A-$  trials. Additionally, the MANOVA revealed that the amplitudes in the Test phase were more negative than those of the Conditioning phase,  $F(1, 37) = 4.82$ ,  $p = .034$ . Finally, a main effect was found for Electrode,  $F(2, 36) = 30.85$ ,  $p < .001$ , and post-hoc analyses again showed that Fz and Cz amplitudes were more negative than Pz amplitudes.

In contrast to the results of the P300 and N200 in response to target stimuli, the results of the RP correspond to our hypothesis concerning effects of occasion setting on target stimuli. According to this hypothesis, responses to  $X \rightarrow A+$  trials should have been more negative than responses to  $Y \rightarrow A-$  trials. This was the case, and it might suggest that an association between the target and the response was formed, which indeed was modulated by the feature stimulus.

As the RP reflects selective attention to an output channel (Brunia, 1993), in the case of the present experiment, attention to preparing for a button-press, it is also possible that participants only actively prepared to make the response as a result of the presentation of the features. Therefore, it does not necessarily mean that the RP reflects occasion setting; it may also be explained by a direct association. That is, after the presentation of the feature, the participants prepared themselves to make the response.

In conclusion, the results of this experiment seem contradictory. If we had only investigated the behavioral responses of the participants, we would have concluded that participants used an occasion-setting strategy. But, the electrophysiological responses can all be explained as suggesting simple associative learning. In order to further investigate if the amplitudes of the P300 and N200 components in response to target stimuli are an important factor in occasion setting, we performed a second experiment, which could not be solved by simple associations.

## **Experiment 2**

In the second experiment, we employed a serial feature-ambiguous discrimination task (e.g., Nakajima, 1998), which, in contrast to Experiment 1, cannot be solved by simple associative strategies, because within one trial, neither the feature, nor the target in isolation, gives conclusive information about the appropriate response. Participants had to learn to respond to target A if it was preceded by a feature X, and not if presented alone. They further had to learn to not respond to target B if it followed X, but to respond to B when it was presented alone ( $X \rightarrow A + A - X \rightarrow B - B +$ ). Possible differential responding to target stimuli was again investigated by means of ERPs.

### **Materials and Methods**

The method was the same as in the first experiment, with the following exceptions.

#### *Participants*

Twenty-six students (seven men, mean age 24 years) participated in the experiment. They were either paid for their participation, or received course credits.

#### *Apparatus*

Only one visual stimulus, the black circle, was used. Besides the 1500-Hz tone, a second auditory stimulus, a 1750-Hz, 70-dB(A) tone was used.

#### *Procedure*

Four different trial types were used. The first type consisted of X that was followed by A ( $X \rightarrow A$ ), the second of A presented alone (A), the third of X followed by B ( $X \rightarrow B$ ), and the fourth of B presented alone (B). Half the participants received the 1500-Hz tone as A and the 1750-Hz tone as B; the relationship was reversed for the other half. The participants had to learn to press a button after the end of a target stimulus during the  $X \rightarrow A$  and B trials ( $X \rightarrow A + B +$ ), but not after A and  $X \rightarrow B$  trials ( $A - X \rightarrow B -$ ). Three blocks of stimuli were presented, with 26 trials of every trial type in each block. The participants received a 5-minute break between trial blocks. All other details were as in Experiment 1.

#### *Data analysis*

Behavioral analysis. The criterion for the task to be considered as learned was that correct responses were made to at least two trials of each type in a row. Button presses in response to  $X \rightarrow A +$  and B+ trials, and no button presses in

response to A- and X→B- trials, were considered as correct. Reaction times (RT) of responses to X→A+ and B+ trials, which were defined as the interval between target offset and the time of the button press, were analyzed for all three blocks. Only RTs of correct responses were analyzed.

EEG analysis. ERPs were calculated in response to the targets (AEP and RP). Because the ERPs of the three blocks did not differ for both features and targets, grand averages were constructed for each trial type (X→A+/A-/X→B-/B+). The data of three participants were excluded because of noisy ERPs.

Statistical analysis of the behavioral and EEG data. RTs were analyzed by means of a MANOVA with Trial Type (X→A+ and B+, 2 levels) and Block (3 levels) as within-subject factors. Because the features were identical on the X→A+ and X→B- trials, the features were not analyzed. The amplitudes of the AEP and RP components were analyzed for the same electrode sites as in Experiment 1. As Block did not significantly affect the amplitudes of the ERP components, MANOVAs were performed with Trial type (X→A+, A-, X→B, and B+) as within-subject factor.

## Results and Discussion

### *Behavioral analysis*

Five participants did not reach the criterion before the end of the first block. They were excluded from further analysis. The ANOVA on the RTs of the X→A+ and B+ trials revealed an interaction between Trial type and Block,  $F(2, 16) = 8.3$ ,  $p = .003$ . A subsequent ANOVA on separate blocks revealed that in Blocks 2 and 3, reaction times did not differ between X→A+ and B+ trials. The reaction times, however, did differ in Block 1,  $F(1, 17) = 5.38$ ,  $p = .033$ . The RT was shorter on X→A+ (362 ms) than on B+ (400 ms) trials.

### *ERP overview*

Figure 3 shows the AEPs in response to the targets for the Fz, Cz, and Pz sites and the mean amplitudes of the P300, N200, and RP components are presented in Table 2. It can be seen that a large P300 is present at Pz in response to the target stimuli, whereas the N200 component is best visible at Fz. Additionally, a RP can be observed at Fz and Cz. This RP shows more negativity in response to X→A+ and B+ trials than to X→B- and A- trials.

### AEP

The MANOVA on the amplitudes of the auditory P300 component showed a main effect of Electrode,  $F(2, 16) = 21.18, p < .001$ . The amplitudes were more positive at Pz than at Cz and Fz, and the amplitudes were more positive at Cz compared to Fz.

A MANOVA on the N200 component amplitude also showed an effect of Electrode  $F(2, 16) = 18.52, p < .001$ . The amplitudes of the N200 component were more negative at Fz than at Cz and Pz.

No significant effects were found for target stimuli with regard to Trial type. According to our hypothesis in Experiment 1, this might be an indication of a simple association. The current task could, however, not be solved by means of simple conditioning. This suggests that the P300 and N200 components are not differentially affected at target stimuli in feature discrimination tasks.

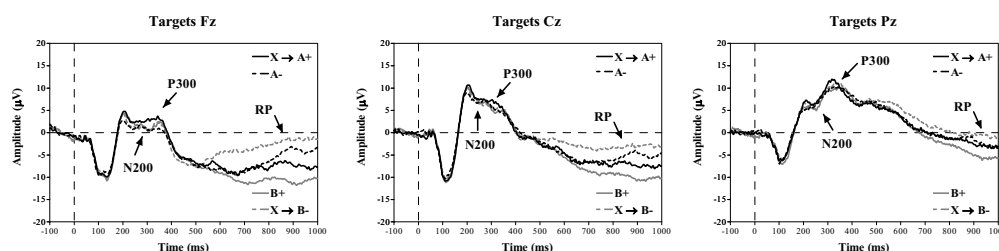


Figure 3. Grand average ERPs in response to the target stimuli in Experiment 2. The three panels show the targets in response to the  $X \rightarrow A+$  (black lines),  $A-$  (black dotted lines),  $B+$  (grey lines), and  $X \rightarrow B-$  (grey dotted lines) trials for the Fz, Cz, and Pz sites.

### RP

Statistical analysis of the mean amplitudes of the RP showed an interaction between Trial type and Electrode,  $F(6, 12) = 3.25, p = .039$ . The amplitudes at the Fz and Cz site were more negative than those of the Pz site. It further showed a main effect of Trial Type,  $F(3, 15) = 4.95, p = .014$ , which reflected that  $X \rightarrow A+$  trials elicited more negative amplitudes than did  $X \rightarrow B-$  trials, and  $B+$  trials elicited more negative values than did both  $A-$  and  $X \rightarrow B-$  trials.

As participants could not solve the task of Experiment 2 by simple associations, and as we again found effects on the RP in Experiment 2, the task effects on the RP component in Experiment 1 may suggest that it reflected the operation of occasion setting in this experiment too.



## **General Discussion**

In the present study, we investigated the electrophysiological correlates of occasion setting, as observed in feature discrimination procedures. Furthermore, we attempted to assess whether responding indeed depends on occasion setting, or rather on simple associations. In the first experiment, participants had to learn to respond to a target stimulus (A) if it was preceded by feature X, and to not respond to that target when presented after feature Y. After a counter-conditioning procedure, in which participants had to respond to Y-alone, but not to X-alone presentations, a test phase was implemented to see whether participants responded in the same way as they did in the first, or the second phase. In the second experiment, participants had to learn to respond to target A if it was followed by feature X, and to not respond after single presentations of A. Moreover, they had to learn to not respond to target B if it was followed by X, but to respond to B when it was presented alone ( $X \rightarrow A+/A-/X \rightarrow B-/B+$ ).

## **Electrophysiological correlates**

The behavioral results of Experiment 1 showed that, in Phase 3, participants responded to A after X, and not to A after Y, as in Phase 1. The ERP results showed marginally enlarged P300 amplitudes in response to feature X compared to feature Y in the conditioning and test phases, but no feature effects on the N200 component in these phases. No differential responding to the target stimuli with regard to the P300 component was found, but it was found with regard to the N200 at Pz in Phase 1, and to the Readiness Potential in both Phases 1 and 3.

In Experiment 2, the amplitudes of the P300 component in response to target stimuli in were not affected by the task manipulation, as in Experiment 1. Furthermore, although the N200 did differ between  $X \rightarrow A+$  and  $Y \rightarrow A-$  trials in Experiment 1, no effect on the amplitude of the N200 was found in Experiment 2. The RP, however, again showed task effects. The amplitudes were more negative at  $X \rightarrow A+$  and  $B+$  trials than at  $A-$  and  $X \rightarrow B-$  trials.

A question that arises is why we did not find any effect on the P300 component in response to targets, although it is a component affected in tasks like the CPT or Go/NoGo tasks. A reason may be that typical 'P300 effects' are usually found in tasks in which the different stimuli have different probabilities (e.g., Ochoa & Polich, 2000; Polich & Margala, 1997; Sambeth et al., 2003). In the current experiments, the probabilities of the different trial types were the same, making

all trials equally important, which may have caused differences in P300 amplitude to decrease.

However, a P300 component is only present if a stimulus is task-relevant. If stimuli are presented without the need to listen or to respond to them, a P300 component is absent (e.g., Bourbon, Will, Gary, & Papanicolaou, 1987; Polich, 1989). In the current two experiments, clear P300 components could be determined for targets at Pz, indicating that the targets were fully processed. Possibly, all stimuli were equally important and, therefore, no differences were found between the different trial types. Perhaps the P300 component is not differentially affected by target stimuli in any feature discrimination task, which would imply that is not an electrophysiological correlate of occasion setting. The same holds for the N200 component.

The effect that was present in both experiments was the increased RP at targets that required a response compared to targets to which no response had to be executed. It is, therefore, possible that the RP is one of the electrophysiological correlates of occasion setting.

### **Occasion setting or simple associations?**

One of the possible explanations for the present behavioral and electrophysiological results is that they indeed reflect occasion setting. The participants' behavioral responses were not affected by a counter-conditioning manipulation in the first experiment, which suggests that the feature did obtain modulatory properties and, thus, that an occasion-setting strategy was used. Furthermore, in both experiments the RP was more negative at targets that were followed by a response than at targets that were not followed by a response. This implies that an association was established between the targets and (non)-responding, which was modulated by the features.

However, the amplitudes of the P300 component tended to be larger at presentations of feature X than of feature Y in Phases 1 and 3 of Experiment 1. As hypothesized in Experiment 1, this might be an indication for a direct, simple association between the feature and (non)-responding, rather than for occasion setting. Another result in favor of simple conditioning is, still, the lack of a differential P300 response to targets. We hypothesized that, if occasion setting had occurred, the P300 component should be larger in amplitude at target A following X than at A following Y in Experiment 1.

Yet another explanation of the current results is that offered by Pearce (1987, 1994; Pearce & Bouton, 2001). In Pearce's configural learning approach, the feature and target may be seen as one compound stimulus, and responding depends on the associative strength between that compound and the US, together with any associative strength that generalizes to it from similar compounds that have been conditioned. According to this theory, in Phase 1 of Experiment 1, participants may have learned to respond to one compound ( $X \rightarrow A$ ), but not to the other ( $Y \rightarrow A$ ). In Phase 2, the participants learned that they now had to respond to Y and to not respond to X. Furthermore, they learned that the response requirement was also reversed (response to Y instead of X). However, the high excitatory associative value of feature Y and the inhibitory associative value of feature X established in Phase 2 should generalize to the, respectively,  $Y \rightarrow A$  and  $X \rightarrow A$  stimulus configurations of Phases 1 and 3. This generalization effect should result in a less clear choice on the  $X \rightarrow A$  and  $Y \rightarrow A$  trials in Phase 3 than empirically observed.

One other problem with the configural learning approach is the fact that the reaction times (RT) measured in Experiment 2 differed between  $X \rightarrow A+$  and B+ trials. If stimuli were seen as one compound, participants would not have known until the start of A or B that they had to make a response, and the RTs should have been equal. However, the X in the  $X \rightarrow A$  trials had a facilitating effect: the RTs were shorter on these trials than on the B-alone trials.

A final, and most likely, explanation for the results may be that both simple associations and occasion setting were established in this study, because we did see both marginally larger P300 amplitudes at features and increased RPs at targets in response to trials to which a response had to be executed. Holland (1992) and Swartzentruber (1995) both proposed that simple excitatory processes and more complex modulatory processes can occur independently and simultaneously. Furthermore, Holland (1992) suggested that single cues might be represented in both a complex conditional memory system and in a more simple memory system. The representation in the conditional memory system causes the cue to acquire modulatory properties, whereas that in the simple system may initiate more simple associations.

Similar to the theoretical assumptions of Holland (1992) and Swartzentruber (1995), and comparable to our results, were the results of Hardwick and Lipp (2000). In a feature-positive discrimination experiment a tactile target was followed by an electric shock after one visual feature, but not followed by the shock if the target was presented alone. Hardwick and Lipp examined whether

auditory startle probes, presented during targets, elicited larger eye blinks if the targets followed features than if the targets were presented alone. This indeed was the case, suggesting modulatory powers of the feature. In a second experiment, in which one group received a feature-positive discrimination and the second group was presented a feature-negative discrimination, they investigated the effects of the features on the auditory startle probes. They found that the feature-positive group elicited larger eye blinks at features than did the feature-negative group. In contrast to Experiment 1, this supports an interpretation in terms of a direct feature-US association, because the feature is directly associated to the unconditioned stimulus. Hardwick and Lipp (2000), therefore, concluded that the responses recorded during the features and targets reflected two different associations: simple associations and occasion setting.

### **Retrospective versus prospective coding strategy**

Besides that the P300 effects in response to features can be explained by simple learning in our study, the effect may have been caused by a so-called prospective coding strategy. From matching-to-sample paradigms, two different styles have been proposed, namely a retrospective and the prospective coding strategy (Swartzentruber, 1998). In the former case, after the presentation of the feature, the subject keeps a representation of this feature passively in working memory. At the time the target is presented, a choice is made according to the contents of working memory. In the case of a prospective coding strategy, the presentation of the feature generates an associatively generated expectation of the response that will have to be made after the presentation of a target. In the prospective coding strategy, the feature plays an active role during the trial, whereas the feature is a rather passive stimulus in the retrospective coding strategy.

The notion of prospective coding strategies could explain the effects in Experiment 1, in which we found marginally significant differential effects on the P300 amplitudes at feature stimuli. The feature might have played an active role. One indication that participants indeed used a prospective coding strategy comes from the analysis of the reaction times in Experiment 2. The reaction times were shorter at  $X \rightarrow A+$  than at  $B+$  trials. The feature thus had a response-facilitating effect, suggesting an active role for the feature during each trial.

## **Conclusions**

In conclusion, the results of the present study suggest that the P300 and N200 components are no electrophysiological correlates of occasion setting, whereas the RP component is one of them. Despite the absence of a clear task effect on ERP components earlier than about 400 ms, in general, studying all ERP components may be a good tool to further unravel the processes that underlie responding in feature discrimination tasks; ERP components may provide information that may complement information from behavioral responses. Furthermore, the data suggest that occasion setting and simple associations can occur simultaneously and independently, which is in line with several previous findings in both humans and animals.

## **Chapter 8**

# **A comparison of event-related potentials of humans and rats elicited by a serial feature-positive discrimination task**

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*Submitted in revised form*

### **Abstract**

The purpose of this experiment was to compare components of the human and rat auditory event-related potential (ERP) in a serial feature-positive discrimination task. Subjects learned to respond to an auditory target stimulus when it followed a visual feature ( $X \rightarrow A+$ ), but to not respond when it was presented alone ( $A-$ ). Upon solving the task, the N2 component was temporarily more negative in response to the target on  $A-$  than on  $X \rightarrow A+$  trials in both species. However, whereas a P3 component was present in the human participants, this component was absent in the rats. It is suggested that the N2 effect reflects a temporary inhibition process in both humans and rats. Furthermore, both species habituated to the task and/or changed their strategy, causing a decreased amplitude of several ERP components, including the N2.

Descriptors: Auditory event-related potential, discrimination task, human, rat, N2, P3

P50, N100, P200, N200, and P300 (P1, N1, P2, N2, and P3 respectively) components can be distinguished in the human auditory event-related potential (ERP). One frequently studied ERP component is the P3. It is often elicited during an oddball task, in which frequent standard stimuli are interspersed with infrequent target stimuli. The P3 has a larger amplitude following target stimuli than following standard stimuli (see e.g., Ochoa & Polich, 2000; Sambeth et al., 2003 for recent examples), and is generally larger during relevant than irrelevant stimuli (e.g., Kok, 2001; Sambeth et al., 2003). A further frequently studied component in humans is the N2. It is suggested to reflect a non-motor inhibition process and is more negative if a subject has to refrain from responding than when required to make a response (Bokura, Yamaguchi, & Kobayashi, 2001; Bruin & Wijers, 2002; Falkenstein, Hoormann, & Hohnsbein, 1999).

Previous studies have shown P20, N60, P120, and N160 (P1, N1, P2, and N2 respectively) components in rats in response to auditory stimuli (Ehlers, Kaneko, Robledo, & Lopez, 1994; Meeren, Van Cappellen van Walsum, Van Luijtelaar, & Coenen, 2001; Sambeth et al., 2003; Shinba, 1997; Yamaguchi, Globus, & Knight, 1993). A further component found in several rat studies is interpreted as a P300 (P3) component (e.g. Ehlers et al., 1994; Hurlbut, Lubar, & Satterfield, 1987; Shinba, 1997, 1999). Fairly recently, ERP studies using rats have also used oddball tasks to assess whether cognitive processes and corresponding ERP components in rats are similar to those in humans (Brankačk, Seidenbecher, & Müller-Gärtner, 1996; Ehlers et al., 1994; Hurlbut et al., 1987; Sambeth et al., 2003; Shinba, 1997, 1999; Yamaguchi et al., 1993). Some of these studies found a positive component at approximately 300 ms after stimulus onset (Brankačk et al., 1996; Ehlers et al., 1994; Hurlbut et al., 1987; Shinba, 1997, 1999), which was more positive for targets than for standards. Others (Galicía et al., 2000, Yamaguchi et al., 1993), however, found a component with an earlier latency (220-240 ms). It is not yet clear whether these P3-like components reflect the same cognitive processes as the P3 does in humans (Sambeth et al., 2003).

The present experiment directly compared the ERPs of humans and rats. The ERP components elicited in the rat during oddball tasks are relatively well described, but it is not known whether the same components can be found in these animals in other cognitive tasks, and whether they are as comparable as in the case for the rat and human ERP components during oddball tasks. Therefore we used a procedure that, in contrast to the oddball paradigm, has extensively

been studied behaviorally in rats, but much less in humans, namely a serial feature-positive discrimination procedure.

In this procedure, a target stimulus (A) is followed by an unconditioned stimulus (US, e.g. food) when it is preceded by a feature stimulus (X), and is not followed by the US when presented alone. If subjects directly associate the feature with the US, they may be said to use a simple associative strategy to solve the task. If an association is established between the target stimulus and the US, and if this association is modulated by the feature stimulus, participants are said to use an occasion-setting strategy. In this case the feature sets the occasion for the presence of the A-US link. It has been shown that under relatively well-defined circumstances, such as when there is an empty interval between the termination of the feature and the onset of the target, both rats and humans use an occasion-setting strategy (for human studies see Baeyens, Vansteenwegen, Hermans, Vervliet, & Eelen, 2001; Dibbets, Maes, & Vossen, 2002; Hardwick & Lipp, 2000; Sambeth, Maes, & Coenen, submitted; for reviews on animal research see Holland, 1992; Swartzentruber, 1995).

At least two different models have been proposed regarding the nature of the association underlying occasion setting (see Figure 1). In the model described by Holland (1992), the feature X indicates that the target A is to be reinforced. It positively activates the excitatory A-US association (see Figure 1A). The model described by Nelson and Bouton (1997; Bouton & Nelson, 1998) assumes not only excitatory, but also inhibitory activity (see Figure 1B). However, the feature only affects the inhibition process. If feature X precedes A, X inhibits the inhibiting link, thereby primarily leaving the excitatory link intact. If A is presented alone, however, the inhibition process is active and the effect of the excitatory link is attenuated or eliminated. There is still much debate as to the validity of these two, and related, types of models of occasion setting (e.g., Hall, 2002; Swartzentruber, 1995).

In the current feature-positive discrimination task, both humans and rats learned to respond to a target when it was preceded by a feature ( $X \rightarrow A+$ ), and to not respond when the target was presented alone ( $A-$ ). The ERP in response to the target stimulus was analyzed for each trial type. It is important to note that the stimuli and procedure used differed to some extent between the two species. However, former learning research in humans has shown that different procedures can lead to similar or even identical outcomes in humans and rats (Dibbets, Maes, & Vossen, 2000; Dibbets et al., 2002). To the best of our knowledge, only one human ERP study has used a serial feature discrimination



procedure up to now (Sambeth et al., submitted) and no ERP research has been performed at all in animals using these kinds of tasks. However, based on the above mentioned models of occasion setting, and earlier results of human ERP research, several hypotheses can be formulated regarding the N2 and P3 components in the current experiment.



*Figure 1.* Models of the associative structure underlying performance in a serial feature-positive discrimination task. A = target; X = feature; US = unconditioned stimulus; arrows indicate an excitatory association; a blocked line indicates an inhibitory association. A) The model provided by Holland (1992), in which X modulates the excitatory A-US link, leading to more excitation on X→A+ trials compared to A- trials. B) The model described by Bouton and Nelson (1998). By presenting X, the inhibitory A-US association is inhibited, thus canceling inhibition, whereas the inhibitory link is present during A-alone presentations, counteracting the excitatory link.

The human N2 component is related to response inhibition processes (Bokura et al., 2001; Falkenstein et al., 1999). According to the model of Nelson and Bouton (1997; Bouton & Nelson, 1998), inhibition is only active during A-presentations. Therefore, the N2 component must be more negative during these trials than during the X→A+ trials. Thus, if the inhibition model is the most valid, we predict a more negative N2 component in response to A- than to X→A+ trials in humans. As, to our knowledge, response inhibition in relation to ERPs has not been studied in rats before, we can only speculate about whether this effect will be present, and if so, at which ERP component.

The human P3 component is usually more positive during relevant than during irrelevant stimuli. Furthermore, some authors (e.g., Johnson, 1986; Kok, 2001) proposed that the amplitude of the P3 component is affected by factors such as probability, attention, expectancy, or task relevance. According to the model of Holland (1992), excitation is the key factor in occasion setting, and we predict

that the P3 component will be larger in response to the more relevant X→A+ trials than to A- trials, at least in the human participants. Although P3-like components have been found in rats before, we do not know whether they reflect the same cognitive processes as does the P3 component in humans (Sambeth et al., 2003). Therefore, again, no clear hypotheses can be formulated for this component in the present task.

Given the lack of data on the electrophysiological correlates of feature-positive discrimination tasks, no particular hypotheses were formulated about the other human and rat ERP components. However, given the complete lack of ERP data, any result on this subject is welcome and, therefore, all components were analyzed.

## **Method**

### **Subjects**

#### *Humans*

Eleven students (three men and eight women, mean age 23 years) of the University of Nijmegen, The Netherlands, participated in the experiment. They were either paid for their participation, or received course credits. They were only allowed to take part in the study if they were healthy, did not use medication, and had no psychiatric history. Participants who agreed to participate signed an informed consent.

#### *Rats*

Sixteen one-year old male Wistar rats served as the animal subjects. They were maintained on a 12/12-h light-dark cycle with lights off at 8.00 a.m. The animals were singly housed in Plexiglas cages with unlimited access to water. The rats were kept at 85% of their free-feeding body weight ( $379 \text{ g} \pm 34 \text{ g}$ ) by restricted daily feeding. Animals were handled daily during the experiment. The study was performed in accordance with the guidelines of the European Community for the use of experimental animals and approval of the local ethics committee was obtained.

### **Apparatus**

The human participants were tested in a sound-attenuating, dimly lit cubicle (inside dimensions: 2 x 2.2 x 2 m). The participants were seated in a comfortable

chair. A 17" computer monitor, which was used to present auditory and visual stimuli, was placed 1.5 m in front of the participant. A black circle with a radius of 6 cm served as visual stimulus; a 1500-Hz, 70-dB(A) tone served as auditory stimulus. Participants had a response button in their right hand during the experiment.

The animal subjects were trained and tested in a set of eight identical operant boxes in which EEG recordings could be made. Each box measured 25 x 24 x 40 cm. The front and back walls were clear Plexiglas; the right side wall and floor were composed of 3-mm stainless steel rods that were spaced 1.3 cm apart. The top was left open to enable EEG recordings in the freely moving rat. Centered in the aluminum left side wall was a 5 x 5 x 3 cm recessed food magazine to which 45-mg precision food pellets could be delivered. Visits to the food magazine were registered by means of an infrared emitter and sensor. Two LEDs, which produced a green light, were mounted on the left side wall, 6 cm to the left and to right, and 3 cm above the food-magazine. Two speakers were mounted on the left side wall, 6 cm to the left and to the right of the food-magazine. These speakers were used for presenting a 4-kHz tone with an intensity of 70 dB(A). Each box was enclosed in a sound-attenuating chamber containing a printed circuit board, a set of cables, and a swivel for EEG measurements.

### **Electrode placement**

#### *Humans*

EEG activity was recorded using an electrode cap with tin electrodes from the Fz (frontal), Cz (central), and Pz (parietal) sites according to the international 10-20 system, with the right mastoid as reference. Eye movements were detected by horizontal and vertical electro-oculogram (EOG) recordings. Impedance was less than 5 k $\Omega$  for all subjects. EEG and EOG were filtered between 0.016 and 100 Hz and sampled at 512 Hz.

#### *Rats*

A tripolar EEG electrode was implanted epidurally under isoflurane inhalation anaesthesia. The first active lead was inserted near the parietal association cortex (A -3.5 L -2.0 related to bregma) (Paxinos & Watson, 1998). The second active lead and the ground were placed on the cerebellum. Two screws and dental acrylic cement were employed to fix the electrode on the skull surface. EEG was filtered between 0.1 and 100 Hz and sampled at 512 Hz.

## Procedure

### *Humans*

First, EEG and EOG electrodes were attached to the skin. Subsequently, the participants were presented a two-block serial feature-positive discrimination task. The visual stimulus was used as feature (X); the auditory stimulus served as target (A). The visual and auditory stimuli had a duration of 1 s. The time between the end of the feature and the onset of the target was 5 s (ISI). The interval between trials was 10-15 s (ITI). Half the trials consisted of a target that was preceded by feature X ( $X \rightarrow A$ ), whereas the other half consisted of a target (A) presented alone. The participants were required to press a button after the end of the target stimulus on the  $X \rightarrow A$  trials ( $X \rightarrow A+$ ), but not on target-alone trials (A-). After each trial, the participants received feedback on the computer screen about their task performance (correct, incorrect). Each block contained 50 trials of each type. The order of presentation of trial types was random, with the restriction that no more than three trials in succession were of the same type. The participants received a 5-minute break between blocks.

Before the experiment, the human participants received instructions about the fact that stimuli were going to be presented and that they had to learn when they had to press or to not press the button. The participants were further told not to press the button during the presentation of a stimulus, but to wait until the stimulus had ended. The participants sat comfortably in their chair during the experiment and were instructed to keep their eyes focused on the monitor and to sit as still as possible.

### *Rats*

First, the tripolar EEG electrode was implanted epidurally and the rats were allowed to recover for two weeks. This period was followed by a period of food restriction. Next, the animals received two 30-min magazine training sessions in which they learned to retrieve food pellets from the food magazine. Ten food pellets were delivered according to a 3-min variable time schedule in each session. Subsequently, the rats were trained in twelve 120-min sessions on a feature-positive discrimination task, in which they learned to respond to the auditory target stimulus when it was preceded by the visual feature ( $X \rightarrow A+$ ), but not when the target was presented alone (A-). This was achieved by delivering a food pellet after termination of the target on  $X \rightarrow A$  trials, and not delivering a food pellet after A-alone presentations. Both the visual and the auditory stimuli had a duration of 5 s and the ISI was 5 s. The ITI was 1-3 min. Thirty  $X \rightarrow A+$  and 30 A-

trials were presented each day in a random order, with the restriction that no more than two trials in succession were of the same type.

### Data analysis

#### *Behavioral analysis*

For the human participants, the criterion for the task to be considered as learned was correct responding to at least two  $X \rightarrow A+$  and at least two  $A-$  trials in succession. For the rat subjects, a ratio score was calculated by dividing the number of correct responses during targets (magazine visit on  $X \rightarrow A+$  trials; no visit on  $A-$  trials) through the total number trials. If the ratio score of one training day was larger than 0.7, the rat had reached the learning criterion.

#### *EEG analysis*

The human EEG was visually checked off-line for EOG activity and other artifacts. The rat EEG was visually checked for movement artifacts. Trials with artifacts were excluded from analysis for both species. Also excluded were the ERPs associated with incorrect responses, that is, no response to a target on  $X \rightarrow A+$  trials, or a response to the target on  $A-$  trials. The EEG fragments within an epoch of 100 ms before target onset and 500 ms after target onset were averaged for all correct responses, using the 100 ms prestimulus as baseline value.

Separate averages in response to the target stimulus on the two trial types were determined for each individual. Grand averages were constructed for trial types for the human and rat subjects. The data of both species were divided into several smaller blocks in order to investigate whether the amplitude of the ERP components changed in the course of the experiment. The data of the rats were divided into three blocks of four days on the basis of task accomplishment (rats were able to perform well from the start of Block 2, see results section). The data of the human participants were divided into four blocks of 25 trials of each type (2 blocks per large block), also on the basis of task accomplishment (all participants were able to perform well by the end of Block 1, see results section). However, only the first three of the smaller blocks were statistically analyzed. This was done because we did not want to mix up trials from the two sessions and because the results for Blocks 3 and 4 were similar.

*Table 1.* ERP components for targets with corresponding latency ranges for the human and rat subjects

Component	Humans	Rats
P1	P75 (40-90 ms)	P40 (32-47 ms)
N1	N115 (90-140 ms)	N75 (50-100 ms)
P2	P200 (160-240 ms)	P130 (100-155 ms)
N2	N265 (220-310 ms)	N180 (165-200 ms)
P3	P305 (250-360 ms) at Pz	XXX

The peak amplitudes of the ERP components were determined on the basis of the individual and grand average ERPs. Table 1 summarizes the human and rat components that were determined for statistical analysis of the ERP data in response to targets.

#### *Statistical analysis of the EEG data*

Analyses of variance (ANOVAs) were calculated for both the human and rat subjects. Separate analyses were performed for each ERP component with Trial type (X→A+ and A-) and Block as within-subject factors. No ANOVA was performed for the amplitude of the P3 at Cz in humans, because we were uncertain as to whether this was a real P3 component (it was not visible after Block 1). The Bonferroni test was used for post-hoc analyses. The level of significance was set at .05 throughout.

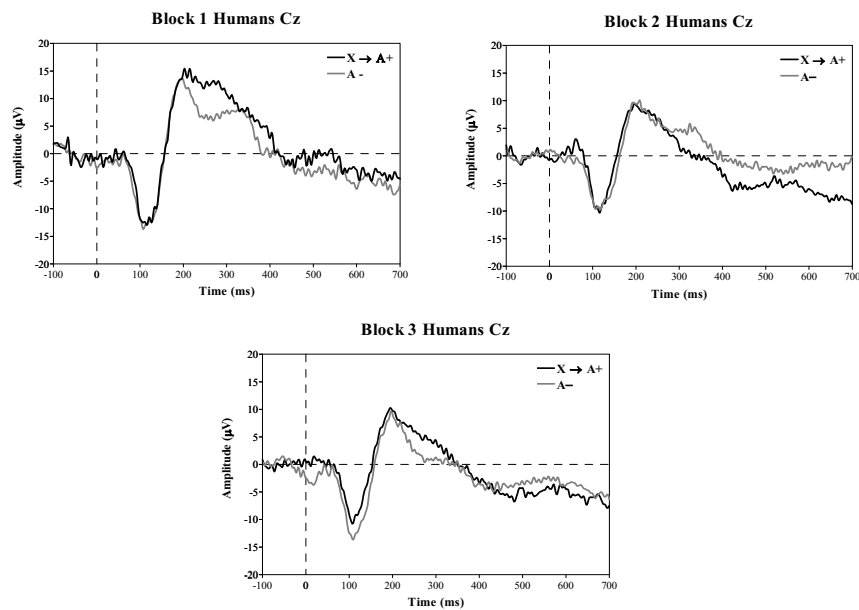
## **Results**

### **Behavioral data: responding to targets**

The human participants reached the learning criterion within the first ten trials. Three rats did not reach the learning criterion before the eighth session of the experiment (end of Block 2). They repeatedly responded to A- trials (false alarms). Therefore, their data were excluded from the analysis. For the other 13 rats, the mean ratio score was .61 during the first sessions of the experiment (Block 1, learning criterion not reached), and .77 and .81 during Blocks 2 and 3, respectively (learning criterion reached).

## Human ERPs in response to targets

Only Cz and Pz electrode sites are shown in the results section, because the analysis of the Fz site did not reveal any additional relevant results. Grand average ERPs at Cz and Pz of Blocks 1 to 3 are shown in Figures 2 and 3, respectively, as elicited by the target. Five ERP components were discerned: P1, N1, P2, N2, and P3. The mean number of trials per participant per block included in the averages (correct responses and good EEG) was 19 for  $X \rightarrow A+$  and 20 for  $A-$  trials. The figures show that the amplitude of the N2 component at Cz and Pz was more negative in Block 1 on  $A-$  than on  $X \rightarrow A+$  trials. Moreover, in Block 1, a P3 was present at Pz on  $X \rightarrow A+$  and  $A-$  trials, although it did not seem to differ at 300-350 ms. In the other blocks, this component had decreased considerably. A final observation is that the amplitudes of the N1, P2, N2, and P3 components decreased in the course of the experiment.



*Figure 2.* Grand average ERPs at Cz of the human participants in three blocks of 25 trials, as evoked by targets preceded by a feature ( $X \rightarrow A+$ , black line) and targets presented alone ( $A-$ , grey line). Latencies are shown on the x-axis in milliseconds and amplitudes are presented on the y-axis in microvolts. Note that the amplitude of the N2 component was more negative in response to targets preceded by a feature than to target presented alone in Block 1.

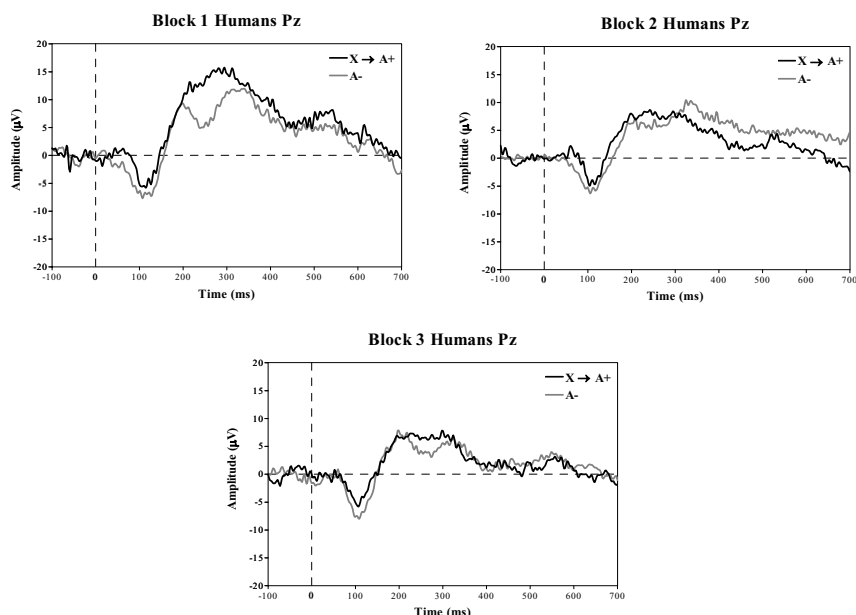


Figure 3. Grand average ERPs at Pz of the human participants in three blocks of 25 trials, as evoked by targets preceded by a feature ( $X \rightarrow A^+$ , black line) and targets presented alone ( $A^-$ , grey line). Note the N2 effect in Block 1.

The ANOVA on the amplitudes of the P1 component revealed a significant main effect of Trial type at Cz,  $F(1, 10) = 6.19$ ,  $p = .032$ , and Pz,  $F(1, 10) = 5.27$ ,  $p = .045$ , but no Block or interaction effects ( $F_s \leq 1.50$ ). The amplitude of the P1 was more positive on  $X \rightarrow A^+$  than on  $A^-$  trials.

An ANOVA on the N1 component amplitude revealed an effect of Block,  $F(2, 9) = 7.64$ ,  $p = .011$ , at Cz only. No other effects were found with regard to this component at any electrode site ( $F_s \leq 1.54$ ). Post-hoc analysis of the Block effect showed that the N1 was more negative in Block 1 than in Block 2 and also more negative in Block 3 than in Block 2. The analysis on the P2 component revealed a main effect of Block at Cz only,  $F(2, 9) = 7.93$ ,  $p = .010$ , and no further significant effects ( $F_s \leq 3.16$ ). Post-hoc analysis showed that the amplitude of the P2 was more positive in Block 1 than in Block 2.

An ANOVA on the amplitudes of the N2 revealed main effects of Trial type at Cz and Pz,  $F(1, 10) = 8.14$ ,  $p = .017$  and  $F(1, 10) = 15.59$ ,  $p = .003$ , respectively. No main Block effect or interaction effect was found at either Cz or Pz ( $F_s \leq 2.59$ ). The amplitude of the N2 was less negative in response to target stimuli on  $X \rightarrow A^+$  than on  $A^-$  trials at both Cz and Pz. As visual inspection suggested that this was



primarily the case for Block 1, we were interested in whether this N2 effect statistically disappeared during the experiment. Therefore, ANOVAs were performed for each block separately. The amplitude of the N2 component proved to be significantly smaller for  $X \rightarrow A+$  than  $A-$  trials in Block 1 at Cz,  $F(1, 10) = 8.27$ ,  $p = .017$ , and Pz,  $F(1, 10) = 11.39$ ,  $p = .007$ , but not in the other blocks ( $F = .20$  and  $3.43$  for Blocks 2 and 3 at Cz, and  $F = 4.46$  and  $3.02$  for Blocks 2 and 3 at Pz).

The ANOVA on the amplitudes of the P3 component at Pz revealed a main effect of Block,  $F(2, 9) = 19.98$ ,  $p < .001$ , but no main effect of Trial type ( $F = 3.13$ ) and no interaction ( $F = .47$ ). Post-hoc analysis of the main Block effect showed that the amplitude of the P3 was larger in Block 1 than in Blocks 2 and 3. Visual inspection of the P3 component suggested that effects of Trial type are also present, namely in Block 1. As the P3 component was important for one of the hypotheses, ANOVAs were performed for each block separately. However, they did not show any significant effects ( $F_s \leq 1.79$ ).

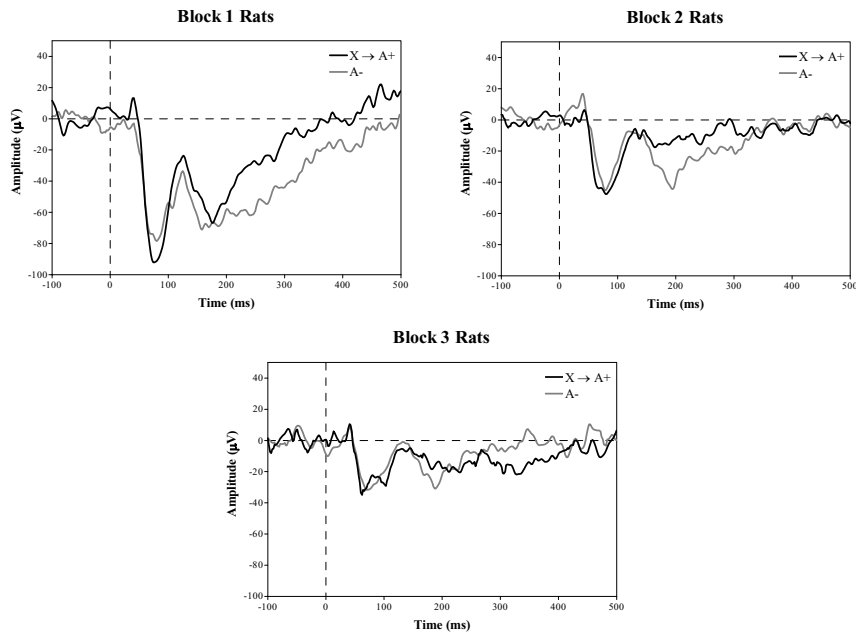
### **Rat ERPs in response to targets**

Grand average ERPs of the rats are shown in Figure 4. This figure shows that four components, with the same order of polarities as found for the human ERPs, were present 40, 75, 130, and 180 ms after stimulus onset. No P3 was detected. The mean number of trials per rat per block included in the averages (correct responses and good EEG) was 93 for  $X \rightarrow A+$  and 66 for  $A-$  trials. The reason for the average of  $X \rightarrow A+$  trials being based on more trials than was the case for  $A-$  trials is that rats made less errors on these former trials. The N2 (at 180 ms) component was more negative in Block 2 in response to targets at  $A-$  than at  $X \rightarrow A+$  trials. Furthermore, the figure shows that the amplitudes of the four ERP components showed a tendency towards baseline (0  $\mu$ V) in the course of the experiment.

An ANOVA on the amplitudes of the P1 component did not reveal any significant effects ( $F_s \leq 1.65$ ). The ANOVA for the N1 component, however, did detect a main effect of Block,  $F(2, 11) = 14.40$ ,  $p = .001$ . No significant main effect of Trial type and no interaction were found with regard to the N1 ( $F_s \leq .34$ ). Post-hoc analysis showed that the amplitude of the N1 was more negative in Block 1 than in Blocks 2 and 3. The ANOVA using the amplitude of the P2 revealed a main effect of Block,  $F(2, 11) = 4.43$ ,  $p = .039$ , but no other significant effects ( $F_s \leq$

.31). The amplitude of this component was more positive in Block 3 than in Block 1.

The ANOVA on the N2 component showed main effects of Trial type,  $F(1, 12) = 5.54$ ,  $p = .036$ , and Block,  $F(2, 11) = 14.01$ ,  $p = .001$ , but no interaction ( $F = 1.05$ ). The amplitude of the N2 component was more negative in response to the target on A- than on  $X \rightarrow A+$  trials. Visual inspection suggested that the effect was only present in Block 2. Therefore, ANOVAs were performed for each block separately, which confirmed this impression ( $F(1, 12) = 5.52$ ,  $p = .037$  for Block 2 and  $F = .51$  and  $2.60$  for Blocks 1 and 3, respectively). Post-hoc analysis of the significant block effect showed that the N2 was more negative in Block 1 than in Blocks 2 and 3.



*Figure 4.* Grand average ERPs of the rat subjects in three 4-session blocks, as evoked by targets preceded by a feature ( $X \rightarrow A+$ , black line) and targets presented alone ( $A-$ , grey line). Note that the N2 amplitude was more negative in response to targets that were preceded by a feature than to targets that were presented alone in Block 2.

## **Discussion**

The aim of the present experiment was to compare ERP components of rats and humans that were subjected to an analogous serial feature-positive discrimination task. More specifically, on the basis of the inhibition hypothesis of the N2 and the excitation hypothesis of the P3 components we were interested in effects on the amplitude of these components. The auditory ERPs elicited by the target stimulus in this task were characterized by the presence of two positive and two negative waves in both humans and rats, albeit with different latencies in the two species. An additional late positive component was found at the human Pz electrode site. The N2 component showed analogous task effects in the two species. In the first block of trials in which the subjects had solved the discrimination task, the amplitude of this component was more negative on A- than on X→A+ trials in both species. Besides a differential P1 in humans (for which we do not have an explanation), no other significant task effects were found in either species. However, regarding the changes in the ERP in the course of the experiment, we again found analogous effects in the two species. The overall shape of the ERP remained the same, but the amplitudes of several components (N1, P2, and P3 in humans; N1, P2, and N2 in rats) became closer to baseline (0  $\mu$ V) with continued training.

We hypothesized that, if the model of Bouton and Nelson (1998) is correct, the N2 component in humans should be more negative for targets on X→A+ than A- trials, which was indeed the case. Furthermore, this effect was most prominent in the first block that the participants had learned the task, and disappeared in the course of the experiment. Similar N2 effects have been related to response inhibition (Bokura et al., 2001; Bruin & Wijers, 2002; Falkenstein et al., 1999), supporting the notion that the N2 reflects response inhibition at targets in response to the A- trials.

As, to our knowledge, response inhibition has not been explicitly investigated in animal ERP research until now, we did not have a clear hypothesis regarding this issue. However, we found that the rat N2 component was similarly affected by the task manipulation as was the human N2. It was more negative at A- than at X→A+ trials, and it was most prominent in the block that the rats had learned the task, Block 2. This strongly suggests that the rat N2 component is related to a response inhibition process as well.

Based on Holland's model (1992), the hypothesis concerning the human P3 component was that the amplitude of the P3 should be larger on X→A+ than A-

trials. In the current study, we could identify a P3 component at Pz, but this component did not show any significant task effects, which suggests that the model is incorrect. However, in so-called continuous performance tests (CPT), which are similar to our feature-positive discrimination procedure, it has been shown that the amplitude of the P3 component is more positive on trials requiring a response than on those that do not (Bokura et al., 2001; Tekok-Kilic, Shucard, & Shucard, 2001). A more detailed analysis of the CPT task, however, reveals important task differences. Presentation of our target A is ambiguous, as exactly the same stimulus either requires a response or not. In case of the CPT task, the stimulus to which a response has to be made is not ambiguous; one requires a response and another requires no response. The joint results suggest that ‘excitation’ (as reflected in the P3) is present on both our trial types, and it is the modulation of inhibition (as reflected in the N2) that is responsible for the differential responding on the two trial types.

P3-like components have been found in rats during oddball tasks (Brankačk et al., 1996; Ehlers et al., 1994; Hurlbut et al., 1987; Sambeth et al., 2003; Shinba, 1997, 1999). Therefore, we expected to find this component in the feature-positive discrimination task as well, which was not the case. The reason for this absence may be as follows. Human research has shown that the amplitude of the P3 component depends on several factors. Two important factors influencing the amplitude are probability and interval between stimuli (e.g., Gonsalvez et al., 1999; Katayama & Polich, 1996; Polich, Ellerson, & Cohen, 1996). Other features are, for example, relevance of a stimulus, expectancy, task difficulty, and task complexity (Donchin, 1981; Johnson, 1986; Kok, 2001). One possibility is that the largest part of the P3 amplitude is determined by probability and interstimulus interval (external factors), whereas a smaller part of the amplitude is affected by the more ‘cognitive’ factors (internal factors). If this were the case, the P3 in rats found during oddball tasks might be due to the external factors, whereas the internal factors are either not present in rats, or occur at another point in time. In this respect it is important to note that Talnov and colleagues (2003), using oddball and single-stimulus tasks, found a negative potential at 150 ms after stimulus onset in the rat hippocampus only at long interstimulus intervals and low probability targets. This negative potential was entirely absent at short intervals and high probability targets. It is, therefore, entirely plausible that the cortically visible P3 in rats behaves in similar ways, and was absent in the present study as a result of equal stimulus probabilities.

Based on the current results, one could conclude that the outlined inhibition model is more suitable than is the excitation model. However, some qualifications must be made. The N2 task effect was only temporary in both species. This can either reflect that the subjects switched from an occasion-setting strategy to a simple associative strategy after learning the task, or that they habituated, or both. In unpublished data we found some evidence in favor of the former suggestion: the P300 response to the feature remained stable throughout a serial feature-positive discrimination task, although the P300 amplitude decreased in a no-task condition. This indicates that features are continuously processed throughout a feature-positive discrimination task, whereas the targets are not (the amplitudes decrease). The amplitudes of several ERP components of both humans (N1, P2, and P3) and rats (N1, P2, N2) showed a tendency towards baseline (0  $\mu$ V). The ERPs of the two species, thus, behaved similarly during the task. It is very likely that these amplitude decrements reflect habituation. The amplitude of ERP components in both humans (e.g., N100, P200, and P300) and rats (e.g. P17, N40) have been found to decrease with repeated presentations in many studies (e.g., Bourbon, Will, Gary, & Papanicolaou, 1987; Bruin, Kenemans, Verbaten, & Van der Heijden, 2000; De Bruin et al., 2001; Quian Quiroga & Van Luitelaar, 2002; Ravden & Polich, 1999).

In conclusion, the present study revealed several remarkable similarities with regard to the ERP components of humans and rats in a feature-positive discrimination task, specifically for the N2 component. These results suggest strong similarities in cognitive processes in rats and humans, as was also found in a previous study (Sambeth et al., 2003). The present study further illustrates how ERP research can help us unravel the nature of cognitive processes elicited by these kinds of tasks.

## General Discussion

Little comparative research has been performed on the electrophysiological correlates of learning, although such research may be used to develop, test, and improve models of cognitive processing and, furthermore, give insight into the physiological basis of cognitive processes in general and, in a later stage, cognitive dysfunctions. For this purpose, correspondences between the human and rat event-related potential components were mapped using different kinds of learning paradigms. Additionally, it was intended to relate these components to cognitive processes as observed in learning. Three learning paradigms were used: habituation, discrimination learning, and feature discrimination learning. First, the comparability of the human and rat ERP components during these three paradigms will be discussed. Subsequently, the functional significance of some of the human and rat ERP components is discussed. This is followed by conclusions and remarks.

Generally, the cortically elicited ERPs were characterized by the presence of two positive and two negative ERP components in both humans and rats, albeit with much shorter latencies in the rats than in the humans. This can be seen in Chapters 2, 4, and 8. In humans, a third positive component was present (see Chapters 3, 4, 5, 6, 7, and 8), which could only be identified in Chapter 4 in the rats. This component revealed a similar latency in both humans and rats.

It has to be noted that the latencies of the ERP components of the two species differed to some extent between the various experiments, which was probably due to inter-individual variability and differences in task demands.

### **Learning paradigms**

#### **Habituation**

In the first experiment, it was examined whether short-term and long-term habituation, as well as enhanced re-habituation, can be demonstrated by one or more of the human and rat ERP components. The results in Chapter 2 showed that the amplitudes of several ERP components decreased within a block of stimuli in both humans and rats. This may reflect short-term habituation and/or a recovery-cycle effect. Furthermore, across-block amplitude decrements were also found for some human and rat ERP components, reflecting long-term

habituation (see also Table 1 on p. 127). Additionally, in both species, short-term effects were more pronounced for the early ERP components, whereas long-term habituation was primarily demonstrated for the later components. Long-term effects were also shown in Chapter 8, in which the ERP components showed decreased amplitudes in both humans and rats in the course of a feature discrimination task, which might reflect long-term habituation. The results of these two chapters suggest a correspondence between the human and rat ERP components during repeated stimulation.

Short-term amplitude decrements have also been shown in previous human and rat studies (Bourbon, Will, Gary, & Papanicolaou, 1987; De Bruin et al., 2001; Lutzenberger Schandry, & Birbaumer, 1979; Quian Quiroga & Van Luitelaar, 2002). Long-term habituation has, however, only been observed in human studies so far (Pan, Takeshita, & Morimoto, 2000; Polich, 1989). The long-term effects in rats, therefore, give further insight into the correspondence of the ERP of both species.

Besides the above described short-term and long-term habituation effects, the human results also demonstrated enhanced re-habituation at the P3a component. This means that, after spontaneous recovery, the amplitude of the P3a component habituated faster in a session compared to a previous one. This effect was, however, not present in any of the ERP components of the rats. Enhanced re-habituation is actually a form of long-term habituation, as it implies a combination of short-term habituation and repeated habituation sessions. Therefore, enhanced re-habituation should only occur at ERP components that also demonstrate long-term habituation more directly. In humans these were the N2 (Chapter 2) and P3a (Chapter 3) components, in rats these were the P2 and N2 components (Chapter 2). Figures 2 and 3 of Chapter 2 and Figure 2 of Chapter 3 show that the amplitude of the human components decreased faster both within and between blocks than did the rat P2 and N2. It is possible that the reason for the absence of enhanced re-habituation in rats is related to the fact that the rat ERP decreased more slowly in general.

In sum, even if enhanced re-habituation was not present in the rats, this experiment revealed notable correspondences between the human and rat ERP components.

### *Comparability of the tasks?*

One important aspect must be taken into account when comparing the ERPs of humans and rats: the tasks that are used in the two species must be as

comparable as possible. In the habituation experiment, the stimuli presented to both the humans and rats had a duration of 1 s and were presented with an inter-stimulus interval of 5-10 s. Furthermore, the time between blocks was 5 min in both species. This suggests that the task was as equal as possible.

One aspect of the task was, however, not equal, namely the auditory stimulus frequency. Rats have different auditory thresholds than humans do. Rats are very sensitivity to high pitch tones, whereas humans are sensitive to low pitch tones. Therefore, appropriate tasks will always have to use high pitch stimuli for rats and low pitch stimuli for humans.

### **Discrimination learning**

The second relatively simple learning paradigm that was studied in this thesis is discrimination learning. In the experiment that is described in Chapter 4, the human participants were first instructed to press a button in response to infrequent targets in an oddball task, but not to frequent standards. The rats, however, had to learn by trial-and-error to respond to the targets in the oddball task. Next, both species were presented with tones in a single-stimulus task without the possibility to make the previous response. The ERP at cortical electrode positions in both humans and rats showed a P3-like component that was more positive in response to the targets than to the standards (see Table 1). Furthermore, in humans the P3 was larger on target and standard trials than on the tone in single-stimulus task trials.

An oddball task, in which now humans had to learn to respond to the targets, was performed in the next experiment (Chapter 5). The trials at the start of the experiment were compared to the trials at the end, in order to observe possible effects of learning on the ERP. It was shown that the N1 and P3 components were more positive at targets than at standards at the end of the experiment, whereas the amplitude of these components were equally large at the two trial types at the start.

It is common to find enlarged P3 components in humans in response to targets compared to standards (e.g., Katayama & Polich, 1996; Ochoa & Polich, 2000; Rockstroh et al., 1996). Previous research has also demonstrated the ‘oddball’ effect on the P3 component in rats (e.g., Ehlers, Kaneko, Robledo, & Lopez, 1994; Jodo, Takeuchi, & Kayama, 1995; Shinba, 1997, 1999). An effect that has only been demonstrated before for the rat N2 (N150) and P3 component (Takeuchi et al., 2000), but not for any human ERP component, is that the amplitude



differences between targets and standards need time to develop during a learning experiment. In Chapter 5, it was shown that the effect on the human P3 component was only present by the end of the experiment, but not at the start. This indicates that the effects indeed developed in the course of this study. In this respect, it must be noted that it is usual to instruct human participants before an experiment, as was done in Chapter 4. Therefore, the target is made relevant from the onset and, thus, immediately elicits larger P3 components than does the standard.

In sum, the simple discrimination studies revealed pronounced similarities in information processing with respect to the P3 component.

### *Comparability of the tasks?*

Compared to the habituation study, in which all essential factors were very comparable, the discrimination task in Chapter 4 was less comparable between the two species. In contrast to the habituation experiment, both species had to make a response. In the present study, it was chosen to instruct the humans about the task, whereas the rats had to find out by trial-and-error when they had to respond to a stimulus. However, this may have had a differential effect on the ERP components of the two species. Therefore, the human participants had to learn the task by trial-and-error in the second discrimination task (see Chapter 5) and it was shown that the results were fairly equal in the two experiments. The amplitudes of the ERP components in response to the target stimuli were larger than those to the standards.

A further point that was not completely equal in the first discrimination task is the inter-stimulus interval used. Whereas the interval was 3-5 s for the humans, the interval was 1-3 min for the rats. It was hypothesized that, if the interval was rather long in the animals, they would not make too many false alarms (look for food when none is delivered). In the second discrimination task, the intervals between stimuli were increased and similar results to the first discrimination experiment were found. Thus, interval did not differentially affect the ERPs of humans and rats in the first discrimination task.

A last factor that differed between the humans and rats in the first discrimination task (see Chapter 4) is the motivation of the subjects. The rats were probably more motivated to perform the task, because they were rewarded with food. The humans, on the other hand, knew that they would earn credit points or money, even if they did not perform well on the task. In the second discrimination task (see Chapter 5), the motivation was more similar to that of

the rats in the first discrimination task by having the humans earn points by means of a counter only if they responded properly. However, humans were rewarded with points at both the target and standard stimuli, whereas the rats only received a food reward at target stimuli in Chapter 4. It is not clear to what extent this may have differentially affected the ERPs of humans and rats.

In all, the comparability of the task in the first discrimination experiment was far from optimal, although the second discrimination study in humans only showed that it did not affect the results.

### **Feature discrimination learning**

The most complex learning paradigm that was examined in this thesis is feature discrimination learning. First, two feature discrimination tasks were examined in humans only (see Chapter 7), as these kinds of tasks had not been performed very often so far, and because it is not known whether the cognitive processes in this task are similar between humans and rats. The results revealed no significant effects for the P1, N1, P2, N2, and P3 components, although the behavioral responses did indicate that the participants had learned the task (see Table 1). Effects were, however, found at the Readiness Potential (RP). It was more negative on targets to which the participants had to respond than on targets to which they did not have to respond.

Since the P3 was not differentially affected by the feature discrimination task, it is possible that the P3 component is not one of the electrophysiological correlates of these kinds of tasks. The amplitude of the P3 usually differs between trials as a result of differences in task relevance (e.g., Bokura, Yamaguchi, & Kobayashi, 2001; Johnson, 1986; Kok, 2002; Polich & Margala, 1997). Therefore, a possibility is that all target stimuli were equally relevant in this discrimination study, resulting in equally large P3 amplitudes.

The RP was more negative in response to the targets that were preceded by a feature than to the targets that were presented alone. This suggests that the RP, and thus cognitively preparing to make a response, is one of the electrophysiological correlates of a feature discrimination task.

The next experiment, described in Chapter 8, aimed at comparing the effects of a feature discrimination task between humans and rats. Both species had to learn to respond to a target if it was preceded by a feature, but not if it was presented alone. As in the habituation experiment, no P3 component was present in the animals, whereas it was in humans. However, like in the first feature

discrimination task (see Chapter 7), the P3 was not differentially affected by the target stimuli. An ERP component that was affected in both species was the N2 component. At the time that the subjects had learned the task, the N2 was more negative in response to the targets that were presented alone.

The lack of an N2 effect in the human feature discrimination task of Chapter 7 may be due to the averaging procedure. Averaged ERPs of several blocks were constructed in the experiment described in Chapter 8 and showed that the N2 effect was only temporary. It was present in the first block in which the subjects had learned to respond correctly to the trials, and decreased dramatically thereafter. A grand average of all data was constructed for the data described in Chapter 7, because less stimuli as was the case in the study described in Chapter 8 were presented. This may have decreased the difference in N2 between the different trials, resulting in a non-significant effect.

The human N2 component has been associated with response inhibition (Bokura et al., 2001; Bruin & Wijers, 2002; Falkenstein, Hoormann, & Hohnsbein, 1999; Fox, Michie, Wynne, & Maybery, 2000; Kopp, Mattler, Goertz, & Rist, 1996; Naito & Matsumura, 1996). If a participant has to refrain from responding, the N2 component will be more negative than if the participant has to make a response. The N2 was indeed most negative on targets that did not require a response. It is, therefore, suggested that response inhibition may play a role in feature discrimination tasks in both humans and rats. The N2 effect was only present at the start of the experiment and decreased thereafter. It is possible that inhibition played a role at the start of the experiment, whereas the stimuli were processed more automatically at the end of the experiment.

In sum, the data of the human and rat subjects show remarkable similarities in the feature discrimination task. Thus, not only in simple discriminations, but also in more complex discriminations, are human and rat ERPs relatively similar.

### *Comparability of the tasks?*

The differences in the tasks of the humans and rats were as follows. As in the other tasks, the frequencies of the auditory stimuli differed between the two species in the feature discrimination task described in Chapter 8. The results of the discrimination tasks (see Chapters 4 and 5) indicated that differences in inter-stimulus interval in the tasks of the humans and rats do not differentially affect the ERPs of the two species. Therefore, relatively long inter-stimulus intervals were used in the animals compared to those of the humans, because

with long intervals, the rats do not move to the pellet feeder too frequently, which prevents the occurrence of movement artifacts.

As the results of the discrimination task of Chapter 5 revealed effects of learning on the ERP in both species, both the human and rat subjects had to learn by trial-and-error when they had to make a response in the feature discrimination task (see Chapter 8). In this way, possible learning effects on the ERP in a feature discrimination task could be examined.

In all, the tasks that were used in humans and rats in the feature discrimination study made it possible to appropriately compare the ERPs of the two species.

#### *What was learned in feature discrimination tasks?*

Besides the comparability of the human and rat ERP, it was intended to relate the ERP components to the cognitive processes that take place during a task. According to existing occasion-setting models (e.g., Holland, 1992; Nelson & Bouton, 1997; Swartzentruber, 1995), the associations that are established during feature discrimination tasks are as follows. Associations are formed between the target and an unconditioned stimulus or response (US/UR). This association in turn is modulated by the feature stimulus. The feature, thus, sets the occasion for the target-US link. The configural learning theory (Pearce, 1987, 1994), on the other hand, assumes that the feature and target stimulus are seen as one compound. Responding depends on the association between the compound and the US. A last possibility is that direct associations are established between the feature and the US.

In Chapter 7, behavioral responding, as measured by the reaction time of a button press, revealed that the feature and target stimuli are not perceived as one compound stimulus. The reaction time was shorter on targets that were preceded by a feature than on targets that were presented alone. If the feature and target had been seen as one compound, the participants could not have known until the start of the target whether or not they had to make a response, and, the reaction time would have been equal between the two trial types. This is evidence against the configural learning theory of Pearce (1987, 1994, Pearce & Bouton, 2001).

The effects on behavioral responding and the RP in humans in Chapter 7 and the effects on the human and rat N2 component in Chapter 8 suggest that the subjects used an occasion-setting strategy. This is because the RP and N2 components significantly differed between the different trials types. Nelson and Bouton (1997; Bouton & Nelson, 1998) suggested that in occasion setting, the

association between the target and US is both excitatory and inhibitory. The feature only affects the inhibitory link; it inhibits the inhibitory link, leaving the excitatory link intact. If the target is presented alone, the inhibition process is active. As the inhibition component, the N2, was most negative in response to the targets that were presented alone, the model of Nelson and Bouton (1997; Bouton & Nelson, 1998) can account for the present results. It is, therefore, suggested that modulation of an inhibitory link is responsible for the differential responding in a feature discrimination task.

Besides evidence in favor of occasion setting, evidence in favor of direct associations was also found. The amplitude of the P3 component in response to the targets did not significantly differ between the trial types, but the P3 in response to the features did marginally differ (see Chapter 7). Furthermore, the N2 effect in Chapter 8 was only temporary, as it decreased after the subjects had learned the task. It is possible that both direct associations and occasion setting occur independently at the same time (Holland, 1992). Therefore, it is suggested that an occasion-setting strategy was replaced by a strategy based on direct associations in both humans and rats.

### **The ERP components**

A general remark needs to be made about the functional significance of the ERP components in humans. It is common to instruct the participants about the task requirements. In all but one study described in this thesis, however, the participants had to learn when they had to press or not press a button. It is possible that the ERP components that were found in the humans may not be functionally equal to other task situations. The conclusions that will be made in the following paragraphs may, therefore, be limited to tasks explicitly requiring learning.

#### **P50/P1**

The results regarding the P1 component in humans were as follows (see also Table 1). The P1 did not habituate (Chapter 2) and the amplitude of the P1 did not differ between targets and standards in an oddball task (Chapter 4). However, the P1 was larger at target trials that were preceded by a feature than at target-alone trials (Chapter 8).

*Table 1.* Summary of the significant results of the human and rat ERP components in the present experiments.

		Humans	Rats
Habituation	Chapter 2	P1: no effects	P1: short-term
		N1: short-term	N1: short-term
		P2: short-term	P2: short-term
		N2: short-term and long-term	N2: long-term
	Chapter 3	P3a: enhanced re-habituation	XXX
Discrimination learning	Chapter 4	P1: no effects	P1: no effects
		N1: target > passive	N1: no effects
		P2: no effects	P2: no effects
		N2: target + standard < passive	N2: no effects
		P3: target > standard > passive	P3: target > standard
	Chapter 5	N1: target > standard P3: target > standard	XXX
Feature discrimination learning	Chapter 6	P3: long ISI > oddball and short ISI	XXX
	Chapter 7	N2: no effects	XXX
		P3: no effects	
		RP: $X \rightarrow A+ > Y \rightarrow A-$	
	Chapter 8	P1: $X \rightarrow A+ > A-$	P1: no effects
		N1: blocks 1 + 3 > block 2	N1: block 1 > blocks 2 + 3
		P2: block 1 > block 2	P2: block 3 > block 1
		N2: $A- > X \rightarrow A+$ , in block 1 P3: block 1 > blocks 2 + 3	N2: $A- > X \rightarrow A+$ , in block 2

It has been suggested that the P1 reflects a pre-attentive process that is not sensitive to cognitive operations (e.g., Coles & Rugg, 1995). This means that the P1 is affected by the external properties of a stimulus such as its frequency or intensity, rather than the internal properties such as attention or task relevance. However, the fact that the P1 was differentially affected by the feature

discrimination task indicates that some cognitive process is involved in P1 generation, as is also suggested by Kho and colleagues (2003).

In the feature discrimination task of Chapter 8, the feature signified the presentation of the target, whereas no signal was given before the target in the target-alone trials. It is possible that the feature advanced the processing that is normally reflected by the N1 component (see below). This in turn would have led to the enlarged P1 at targets that were preceded by the feature.

### *Rat equivalent of the human P1?*

A fair amount of research has been dedicated to discover the rat equivalent of the human P1 component that is elicited in the sensory gating paradigm (e.g., Adler, Rose, & Freedman, 1986; Bickford-Wimer et al., 1990; Boutros, Bonnet, Millana, & Liu, 1997; Boutros, Uretsky, Bernston, & Bornstein, 1994; De Bruin et al., 2001; Miyazato, Skinner, Crews, Williams, & Garcia-Rill, 2000; Miyazato, Skinner, & Garcia-Rill, 1999; Stevens, Fuller, & Rose, 1991). Several components have been suggested to be the equivalent, namely the P13 (Miyazato et al., 1999, 2000), P17 (De Bruin et al., 2001), N20-30 (De Bruin et al., 2001; Stevens et al., 1991), and N40-50 (Adler et al., 1986; Bickford-Wimer et al., 1990; Boutros et al., 1994, 1997; De Bruin et al., 2001) components.

In the present experiments, the human P1 did not habituate (see Chapters 2 and 8) and it was only differentially affected in the feature discrimination task. The equivalent in rats should, at best, show results equal to the human P1. However, none of the rat ERP components showed an effect equal to that of the human P1 in the feature discrimination task. Furthermore, all rat ERP components demonstrated either STH or LTH in the habituation experiment, whereas the human P1 did not. Therefore, no suggestions can be made on the basis of the present studies as to which of the rat ERP components is the equivalent of the human P1 component.

### **N100/N1**

Regarding the human N1 component (see also Table 1), it was found that it showed short-term and long-term amplitude decrements with repeated stimulation (Chapters 2 and 8). Furthermore, the N1 was most negative in response to stimuli that required a response in an oddball task (see Chapters 4 and 5). During the feature discrimination task, however, the N1 component was equally negative at the different trials types.

It has been suggested that the N1 component reflects not merely attention, but attention that is invested in the selection of the physical properties of a stimulus (Kok, 1997; Näätänen & Picton, 1987), such as selection of the frequency or intensity of a stimulus. The N1 component was more negative at target than at standard stimuli in the oddball tasks (see Chapters 4 and 5). According to Näätänen and Picton (1987), the target was better attended in these tasks because the participants knew that this was the relevant stimulus frequency. Consequently, the amplitude was enlarged in the present studies. The results of the feature discrimination task (Chapter 8) may be explained accordingly. The two target stimuli were equally attended, because their equal stimulus frequency did not give any information about task relevance. Therefore, the amplitudes did not differ between the two trial types.

In sum, the hypothesis of Näätänen and Picton (1987) can explain the current N1 data, suggesting that the N1 reflects attention that is invested in the selection of the physical stimulus properties.

#### *Rat equivalent of the human N1?*

The rat ERP component that is functionally equivalent to the human N1 should reveal short-term amplitude decrements with repeated stimulation and long-term habituation with repeated stimulus blocks, and further be more negative in response to stimuli in discrimination tasks that require a response than to those that do not. The component in rats demonstrating part of these effects is the N1 component. It showed short-term decrements in the habituation study and long-term habituation in the feature discrimination task. However, the rat N1 was not differentially affected by any of the task manipulations. The component that did reveal task effects in a discrimination task (see Chapter 4) is the rat P3 component. However, this component was not even present in the habituation studies. Thus, none of the rat ERP components demonstrate equal effects to the human N1 component.

Galicia and colleagues (2000) have shown in rats that, besides the P3, the N1 component too is larger in response to targets than to standards in an oddball task. A close look at the figures in Chapter 4 shows that, in this experiment, the N1 was more negative in response to the trials that required a response than to those that did not. However, because of too large variability this effect did not reach a level of statistical significance.

In sum, the tendency of the N1 to be enlarged in response to the target stimuli in discrimination tasks, together with the short-term and long-term habituation



effects, suggests that the N1 in rats may at least share some of the characteristics of the human N1 component.

### **P200/P2**

The effect with regard to the human P2 component that was demonstrated in the current studies is habituation (see Table 1). The habituation study (Chapter 2) revealed short-term amplitude decrements, and long-term habituation was present in the feature discrimination task (Chapter 8). The P2, was however, not differentially affected by the task manipulations in any of the discrimination tasks or the feature discrimination task.

In the general introduction, it was suggested that it is not known what the P2 component means in terms of information processing. It is possible that attention modulates the amplitude of the P2 component (Golob, Pratt, & Starr, 2002), although the P2 component may differ between targets and standards in an oddball task even if the stimuli are not consciously attended (Lang, Kotchoubey, Lutz, & Birbaumer, 1997). The current studies did not reveal any information as to the functional significance of this component.

#### *Rat equivalent of the human P2?*

As the P2 component was only decreased with repeated stimulus presentations, the rat equivalent should demonstrate both short-term and long-term amplitude decrements. The rat N1 and P2 components showed both short-term decrements in the habituation study (see Chapter 2) and long-term habituation in the feature discrimination task (see Chapter 8). The rat P2 further showed long-term habituation in Chapter 2. To the current knowledge, both these components may be functionally equivalent to the human P2.

### **N200/N2**

The present experiments showed that the N2 component habituates (see also Table 1). Both short-term and long-term amplitude decrements were demonstrated in the habituation study (Chapter 2). Furthermore, the N2 was less negative in response to targets and standards in an oddball task compared to the stimulus in a passive single-stimulus task (Chapter 4). A final effect was that, at the start of the experiment, the targets that did not require a response elicited

more negative N2 amplitudes than did the targets that were preceded by a feature and required a response (see Chapter 8).

Recent studies have shown that the N2 component reflects response inhibition (e.g., Bokura et al., 2001; Bruin & Wijers, 2002; Falkenstein et al., 1999; Fox et al., 2000). The amplitude of the N2 is more negative if a participant has to refrain from responding than if he or she has to make a response. The results of the second feature discrimination task (Chapter 8) confirmed this idea, at least for the start of the experiment, because it was indeed shown that the N2 was most negative if the participants had to respond to stimuli.

Other suggestions about the functional significance of the N2 component have also been made. Bruin, Wijers, and Staveren (2001) suggested that it may rather be response activation instead of response inhibition that significantly affects the N2 component, although they did not give any argument for their idea. However, the N2 was more negative during stimuli in a passive single-stimulus task than during targets and standards in an oddball task. The participants knew that they did not have to press a button to any of the stimuli in this passive task, because they were instructed not to press any button. Therefore, the enlarged N2 in this experiment cannot be due to response activation.

In conclusion, the human N2 component is likely to reflect response inhibition.

#### *Rat equivalent of the human N2?*

The rat equivalent of the human N2 should, at best, demonstrate both short-term and long-term amplitude decrements, and further be more negative if the rat does not have to respond to a stimulus than if it has to make a response. The N2 component that was found in the rats in the current experiments displayed several of the human N2 effects. Although the N2 component did not show short-term amplitude decrements within a block of repeated stimuli, it did show long-term habituation (see Chapter 2). Furthermore, the rat N2 was more negative in response to the targets that were presented alone than to the targets that were preceded by a feature (see Chapter 8).

In sum, it is possible that the rat cortical N2 component reflects response inhibition.

**P300/P3a and P3b**

The results regarding the human P3 component are as follows. The P3a component demonstrated short-term amplitude decrements, long-term habituation, and enhanced re-habituation (see Chapter 3). Furthermore, the P3b component was more positive in response to target than to standard stimuli in the oddball tasks of Chapters 4 and 5. Chapter 4 additionally showed that the P3b was more positive during the oddball task than during the passive single-stimulus paradigm. The results of Chapter 5 revealed that long intervals elicited larger P3b components than did shorter intervals. The most complex learning paradigms that were used in the current studies, the feature discrimination tasks (see Chapters 7 and 8), however, did not reveal any P3 effects. The amplitude of the P3b component was equally large at the different trial types.

The amplitude of the P3b has been suggested to be affected by many different factors, for example, task relevance, task complexity, attention, global probability, or inter-stimulus interval (Donchin, 1981; Johnson, 1986; Kok, 2002; Polich, 1990a; Polich, Eischen, & Collins, 1994). The present studies indeed showed that task relevance may have an effect, as the P3b was more positive in response to the relevant targets than to the irrelevant standards (see Chapters 4 and 5), and as this only occurred when the participants knew that the targets were relevant. Besides the differential influence of task relevance, a P3b component is only present if stimuli are task relevant at all. It is absent in no-task situations such as in habituation paradigms (e.g., Bourbon et al., 1987; Polich, 1989). This might suggest that the targets in the feature discrimination tasks were equally relevant, as the P3b component was present, but was not differentially affected by the task manipulations (see Chapters 7 and 8).

The effects that were found with regard to the P3b component can at least partially be explained by another factor as well, namely global probability. In the oddball tasks, the probability of the target stimuli was only 20%, whereas the standards were presented in 80% of the trials. As the amplitude of the P3b increases with decreasing stimulus probability (Johnson & Donchin, 1982; Katayama & Polich, 1996; Polich, 1987; 1990a, 1990b; Squires, Wickens, Squires, & Donchin, 1976), this explains the enlarged P3b amplitude at target stimuli compared to standards. In the second discrimination task (see Chapter 5), the 'oddball' effect developed in the course of learning, that is, the P3 in response to targets was only larger than that to standards at the end of the experiment, when the participants had learned the task, but not at the start. However, the

probability of the targets was 20% from the start of the experiment. This suggests that participants have to learn about the global probability in a non-instruction situation. In the feature discrimination tasks (Chapters 7 and 8), the probability of the different trial types was equal. Therefore, the amplitude should be equally large, which was indeed the case.

Concerning probability, it was shown that inter-stimulus interval (ISI) may even be more important than probability (see Chapter 6). The P3b was more positive in response to the stimuli in a long-ISI condition, in which the ISI was extremely long and stimuli had a probability of 100%, than to targets in an oddball condition, in which the ISI was relatively short and target probability was only 20%. In this respect, Gonsalvez and colleagues (Croft, Gonsalvez, Gabriel, & Barry, 2003; Gonsalvez et al., 1999; Gonsalvez & Polich, 2002) have suggested that the target-to-target interval (TTI) is the determining factor of P3b amplitude. They investigated whether the TTI affects the P3b amplitude independently of ISI and sequence length (the number of standards preceding a target) and found that this was indeed the case.

Gonsalvez and colleagues (Croft et al., 2003; Gonsalvez et al., 1999; Gonsalvez & Polich, 2002) all used short ISIs of up to 4 s to test their TTI hypotheses. Using extremely long ISIs of up to 65 s, it was, however, demonstrated that the amplitude of the P3b may not be affected by TTI per se, but that the ISI itself may have a stronger effect (see Chapter 6). The amplitude of the P3b was more positive in response to stimuli in a long-ISI single-stimulus condition than to targets in an oddball task, although the TTI was equal. The only difference between these tasks was the interspersing of frequent standards between the targets in the oddball task. This caused the difference in ISI between the long-ISI single-stimulus and the oddball conditions.

It seems that time is one of the important factors that influences the amplitude of the P3b. Why does this have such a great influence? It is possible that, with long intervals, the memory of the stimuli has decayed. The mental schema, which scans the information of each stimulus (Donchin, 1981), will, consequently, detect a relatively new stimulus, leading to a revision of the schema and a large P3b amplitude. In the framework of the model of Kok (2002), a large P3b amplitude is elicited with long intervals in the following way. If a stimulus is presented after a long empty interval, it automatically draws attention. Furthermore, the stimulus representation has decayed from memory after this long interval. Attention and working memory together cause a large P3b

amplitude. In both the models of Donchin (1981) and Kok (2002), ‘novelty’, thus, produces the large P3b component.

In the introduction, it was suggested that the model of Kok (2002) includes several important factors that determine the amplitude of the P3b component, such as stimulus probability, task relevance and task difficulty. It was found that ISI may influence the amplitude more than does probability, and that time in general clearly has an effect on the P3b amplitude. Therefore, the factor ‘stimulus probability’ might be extended to ‘probability and time’. It appeared that task relevance had an affect, because targets in an oddball elicited larger P3b amplitudes than did standards. However, as these effects could have been confounded by the factor probability, task relevance should be studied in experiments that apply equal probabilities to all trial types, but different levels of relevance. The third factor, task difficulty, was planned to be manipulated in the present studies. However, even the most complex paradigm that was used, the feature discrimination task, was easy for the participants to learn. Therefore, no conclusions can be drawn about this factor on the basis of the current experiments.

In sum, the P3 component in these studies was affected by ‘probability and time’ and by task relevance, and the model of Kok (2002) provides some useful suggestions about the factors affecting the amplitude of the P3b component in general.

### *Rat equivalent of the human P3?*

The rat ERP component that is functionally equivalent to the human P3 component should be composed of two different components, the P3a and P3b. Furthermore, this component should habituate (see Chapter 3) with repeated stimulus presentations and be more positive in response to the stimuli that require a response than to stimuli that do not (see Chapters 4, 5, and 6), at least in the oddball paradigm.

The rat ERP component that demonstrated an enlarged amplitude in response to targets compared to standards was the P3 component in Chapter 4. However, this component was not even present in the habituation and feature discrimination tasks. One component showed both short-term and long-term amplitude decrements (habituation), namely the P2 component, although the amplitude of this component did not differ between different trials types in either the discrimination task or the feature discrimination task.

Several researchers have suggested that the rat P3 component may be the equivalent of the human P3 (Jodo et al., 1995; Shinba, 1997; Takeuchi et al., 2000, Yamaguchi, Globus, & Knight, 1993), as it shows the usual 'oddball' effect and normally has a latency similar to that of the human P3 component. It was, however, found that the rat P3 was only elicited in an oddball task, whereas a more complex task (see Chapter 8) did not elicit any P3 component. The human P3b component, on the other hand, was still present in the feature discrimination task, although the amplitude was equally positive at both trial types.

It is possible that the rat P3 component is mainly affected by external factors such as probability and ISI, and much less by the more cognitive factors such as task relevance. This would explain why the oddball task in Chapter 4 elicited a P3 component in the rats, as the probability of the targets was less than that of the standards. Furthermore, in the feature discrimination task this component was absent because the two trial types had equal probabilities.

In sum, the rat P3 component may only partly be functionally equivalent to the human P3 component and be affected by factors such as 'probability and time'.

## **Conclusions and remarks**

Comparative research on the electrophysiological correlates of learning can be used to develop a rat model of cognitive processing and, furthermore, may be used to develop, test, and improve models of cognitive functions and dysfunctions. The present studies intended to map correspondences between the human and rat ERP as observed in learning paradigms.

Using a habituation paradigm, it was shown that the relatively early ERP components of humans and rats decreased with repeated stimulation, possibly reflecting short-term habituation. Furthermore, the late components revealed long-term habituation in both species. This suggests a correspondence between the ERP correlates of elemental stimulus processing of humans and rats. The results of the discrimination and feature discrimination tasks applied in both species showed that the rat P3 component may only be partly equivalent to the human P3 component, as it was only present in the discrimination task, but not in the more complex feature discrimination task. However, in the feature discrimination task, the rat N2 component showed effects analogous to the human N2, probably reflecting response inhibition. The results of this task suggest strong similarities in cognitive processes in humans and rats during feature discrimination learning. Overall, the present studies have shown

remarkable correspondences between the ERP correlates of learning in humans and rats.

A few general comments must be made, however, with regard to comparing the ERPs of humans and rats. One important factor is task similarity. If the intention is to study similar cognitive processes in humans and rats, tasks must be as equal as possible in the two species. However, as was shown in the present thesis, the tasks themselves, such as the feature discrimination task, were rather equal between the two species with respect to design and task requirements, but seemed to be more difficult for the rats than for the humans. Future research comparing humans and rats should, therefore, try to develop tasks that both measure the same cognitive processes while being equally difficult.

Secondly, research should try to identify whether the rat ERP components are present at more cortical locations besides the vertex position. This could be used to examine whether two P3 components exist in rats similar to the human P3a and P3b components. This, in turn, might provide information about whether the rat P3 is indeed partly equivalent to the human P3. Furthermore, as, for example, the human N1 shows its most prominent effect at frontal sites in humans, it is possible that some of the rat ERP components will demonstrate task effects at other locations than the vertex position.

In conclusion, the rat indeed showed to be an appropriate animal model for the study of the electrophysiological correlates of cognitive processes, especially with regard to the N2 component. Moreover, the present experiments illustrate that the study of ERP components may be a useful tool to further unravel the nature of cognitive processes underlying performance in learning tasks.

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## Summary

Learning can be defined as a change in capacity for behavior as a result of particular kinds of experience. Learning can be relatively simple, as is the case in habituation or discrimination paradigms, but also rather complex, which is the case in feature discrimination procedures. The brain activity of the learning subject can be examined in order to find out more about the brain processes involved in learning. So far, not much attention has been given to the comparability of the human and rat brain activity under analogous learning conditions. However, by comparing the brain activity of humans and rats, it is possible to gather new information on the functioning of the mammalian brain, which in turn may be used to develop, test, and improve models of cognitive functions and, in a later stadium, models of cognitive dysfunctions. For this purpose, the electrophysiology of humans and rats as induced in different learning paradigms was directly compared.

In *Chapter 1*, the three learning paradigms of habituation, discrimination learning, and feature discrimination learning used in this thesis are introduced. In a habituation task, one and the same stimulus is repeatedly presented and, although the response to the initial stimulus is large, it will decrease with more presentations. In a discrimination task, two or more different stimuli are presented and a subject learns to respond differently to those stimuli, which results in direct stimulus-response relations for each stimulus. An example of a traditional feature discrimination paradigm is as follows. A target stimulus is either preceded by a feature stimulus, or it is presented alone, and the subjects have to learn to respond to the target if it is preceded by the feature. It has been suggested that the feature stimulus sets the occasion for the presence of the target-response link.

The second part of Chapter 1 introduces several event-related potential (ERP) components and discusses their functional significance. ERPs are electrical brain potentials or components that can be elicited by sensory stimuli. The components that have been studied frequently in humans are the N2 and P3 components. The N2 reflects response inhibition, whereas the P3 component is affected by factors such as stimulus probability, task relevance, and task complexity. In rats, a P3 component with characteristics similar to the human P3 has been found.

At the end of Chapter 1, a summary of the literature about ERP studies is given in which one of the three learning paradigms was implemented.

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## **Habituation**

The study described in **Chapter 2** was designed to examine short-term and long-term habituation processes in humans and rats. Both species were presented with four blocks of 25 presentations of an auditory stimulus. Although the latencies of the ERP components differed between humans and rats, two positive and two negative ERP components were found in both species (P1, N1, P2, N2). In both humans and rats, the amplitude of the relatively early ERP components (N1, P2, N2 in humans; N1 and P2 in rats) decreased within a block, reflecting short-term habituation. Furthermore, the late ERP components (N2 in humans; P2 and N2 in rats) decreased between the four blocks of stimuli in both species, indicating long-term habituation. The results suggest a strong correspondence between humans and rats in the ERP correlates of habituation processes.

A further ERP component that was present in the human habituation experiment was discussed in **Chapter 3**, namely the orienting response that is reflected by the P3 component. The amplitude of this component decreased within and between the four blocks of auditory stimuli, reflecting both short-term and long-term habituation. A further effect was that, at the beginning of a new block, the amplitude of the P3 recovered. During the following stimulus presentations, however, the amplitude of the P3 decreased faster than it did in the previous block. This phenomenon is a characteristic of habituation and is called enhanced re-habituation.

## **Discrimination learning**

The following series of experiments were performed to examine the ERP components of both humans and rats in discrimination tasks. In **Chapter 4**, both species had to perform a so-called oddball task that was followed by a passive single-stimulus paradigm. In the oddball task, frequent standard stimuli were interspersed with infrequent target stimuli and the humans were instructed to press a button in response to the targets, whereas the rats had to learn to visit a pellet feeder at target stimuli in order to receive a food pellet. In the passive task, only frequent standards were presented and none of the two species had to respond to those stimuli. As in the habituation experiment, two positive and two negative components were present in both humans and rats. Additionally, a P3 component was found in both species, which was more positive in response to the target than to the standard stimuli. This suggests similarities in processing of

stimuli in discrimination tasks. In humans, the N1 also showed a task effect. It was more negative in response to target than to the stimuli in the single-stimulus task. In rats, however, the N1 component did not reveal any significant results. This suggests that the ERPs of humans and rats do also differ in discrimination tasks.

A second oddball experiment was performed in which the effects of learning on the human ERP components were examined. This experiment is described in **Chapter 5**. In contrast to the discrimination experiment described in Chapter 4, in which the humans were instructed about the task requirements, they now had to learn to respond to the target stimuli in Chapter 5 by pressing a button. The N1 and P3 components were equally large in response to the target and standard stimuli at the start of the experiment, whereas the target elicited larger N1 and P3 amplitudes compared to the standard at the end of the experiment. The results suggest that learning affects the human ERP.

In **Chapter 6**, an experiment is illustrated in which the factors that affect the human P3 amplitude in a learning experiment are examined. The participants were divided over three conditions. The first group had to learn to press a button in response to target, but not standard stimuli in an oddball task. The inter-stimulus interval (ISI) used in this condition was 9-20 s. The second and third groups had to learn to press a button in response to all stimuli in a single-stimulus task. These two conditions differed only with respect to the ISI used; in the short-ISI group, the ISI was 9-20 s, whereas it was 40-90 s in the long-ISI group. Previous research has shown that target-to-target interval critically determines the amplitude of the P3. In this experiment, it was, however, found that the target-to-target interval may not crucially affect the P3 amplitude. Although the target-to-target interval was equal in the long-ISI single-stimulus and the oddball task, the stimuli in the long-ISI condition elicited larger P3 amplitudes than did the targets in the oddball task. Furthermore, the P3 was equally large in the oddball and short-ISI conditions, whereas the target-to-target interval was not equally large. It was suggested that, especially with long ISI's, ISI may largely determine the amplitude of the P3.

### **Feature discrimination learning**

A last series of experiments studied the ERP components that are elicited in a feature discrimination task. In a traditional feature positive discrimination task, a target stimulus is either preceded by another stimulus, the feature, or it is



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presented alone, and subjects have to learn to respond to the target if it is preceded by the feature. It has been shown in rats that the feature acts as an occasion-setter. This means that it sets the occasion for the presence of a target-response link. As feature discrimination tasks have not been used frequently in humans before, it is not clear which cognitive processes play a role in these kinds of tasks in humans. Therefore, first two feature discrimination tasks were examined in humans in order to assess whether the cognitive processing of humans in these tasks equals that of rats. These experiments are described in **Chapter 7**. In the first experiment, participants had to learn to respond to an auditory target stimulus if it was preceded by a visual feature X, but not when the target was preceded by feature Y. The amplitudes of the N2 and P3 components did not differ between the two target stimulus situations. The Readiness Potential, which is negativity that reflects cognitive motor preparation, however, did show a task effect. It was more negative at the targets to which the participants had to make a response than at the targets that did not require a response. With respect to the feature stimuli, it was found that the P3 component was marginally larger if the participants had to respond to the targets than if the participants did not have to make a response. This suggests that occasion setting occurred together with direct feature-response associations. In the second experiment, participants had to learn to press a button if an auditory target A was preceded by a visual feature and if an auditory target B was presented alone. They learned not to press a button if target A was presented alone or if target B was preceded by the feature. Again, the only significant differences were found for the RP. The targets following a response elicited a more negative RP amplitude than did the targets that were not followed by a response. The results of these two experiments suggest that occasion-setting processes are present in the feature discrimination task, which are reflected by the Readiness Potential. Furthermore, these processes occur together with direct associations.

**Chapter 8** describes a study in which the ERPs of humans and rats in response to the target stimuli in a feature positive discrimination task were directly compared. The humans had to learn to respond to a target if it was preceded by a feature, whereas they learned not to respond if the target was presented alone. The rats had to learn to visit a pellet feeder during the target stimuli that were preceded by the feature in order to get food, but to not visit the pellet feeder at target-alone presentations. Two positive and two negative components were shown in humans and rats. In humans, an additional positive component was present. In both species, the N2 component was more negative if

the subjects did not have to respond to the stimuli, that is, at targets that were presented alone. None of the other human and rat ERP components revealed significant task effects. This suggests strong similarities in cognitive processes in humans and rats in feature discrimination task.

In *Chapter 9*, the results of the present experiments are summarized and discussed. First, the comparability of cognitive processing during the three learning paradigms is discussed. The experiments revealed similarities in cognitive processing as reflected by the ERP components of humans and rats. The relatively early ERP components showed short-term amplitude decrements in both species in the habituation experiment and the later ERP components revealed long-term decrements that reflect habituation (see Chapter 2). Furthermore, the P3 component of humans and rats was enlarged in response to target stimuli compared to standard stimuli in a discrimination task (Chapter 4). Finally, the feature discrimination task (Chapter 8) showed that in both humans and rats, the N2 component was more negative when the subjects did not have to respond than when they did have to make a response.

Second, the functional significance of the human and rat ERP components were discussed. The N2 component has been said to reflect response inhibition. In the feature discrimination task (Chapter 8), it was shown that this may indeed be the case, because this component was more negative if the humans did not have to respond to a stimulus than when they did have to make a response. Furthermore, as the rat N2 component revealed an equal effect, the rat N2 component may share this functionality.

Theoretical models about the human P3 component have suggested that this component reflects event categorization or schema updating and that it is affected by factors such as probability of a stimulus, task relevance, and task complexity. The results described in Chapter 6 suggest that it may not be probability itself that affects the amplitude of the P3 component, but that it may be the time between two stimuli in general. The results of our discrimination experiments (Chapters 4, 5, and 6) are in accordance with the idea that task relevance influences P3 amplitude, because the P3 was always larger in response to the relevant stimuli than to the irrelevant stimuli. The rat P3 component was only present in the first discrimination task (Chapter 4). It is suggested that the rat P3 may share external characteristics of the human P3 such as effects of time and probability, but not the more advanced cognitive characteristics such as task relevance and complexity.

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In conclusion, the N1, N2, and P3 components of rats as elicited during learning tasks share some characteristics with the human N1, N2, and P3 component. This supports the notion of the rat being an appropriate animal model for the study of the electrophysiological correlates of cognitive processes as induced in traditional learning paradigms.

## Samenvatting

Leren kan worden gedefinieerd als een verandering in vermogen tot gedrag als gevolg van bepaalde ervaringen. Leren kan betrekkelijk gemakkelijk zijn, zoals het geval is bij habituatie of discriminatie paradigma's. Het kan echter ook complex zijn, bijvoorbeeld bij feature discriminatie procedures. Om te onderzoeken welke hersenprocessen een rol spelen bij leren, kan de hersenactiviteit van de persoon of het dier dat leert worden gemeten. Tot nu toe is er niet veel aandacht besteed aan de vergelijkbaarheid van de hersenactiviteit van mensen en ratten tijdens verschillende leerprocessen. Echter, dit soort onderzoek zou kunnen bijdragen tot het vergroten van de kennis van cognitieve processen van dieren. Deze kennis kan worden gebruikt om modellen van cognitieve processen te ontwikkelen, testen en verbeteren en wellicht in een later stadium zelfs modellen van cognitief dysfunctioneren te ontwikkelen. Om deze reden werd de hersenactiviteit tussen mens en rat vergeleken tijdens verschillende leer paradigma's.

De drie leer paradigma's zoals gebruikt in dit proefschrift worden besproken in **Hoofdstuk 1**. Dit zijn habituatie, discriminatie leren en feature discriminatie leren. Tijdens een habituatie taak wordt een en dezelfde prikkel of stimulus herhaaldelijk gepresenteerd. De reactie of respons op de eerste stimulus is groot, terwijl deze zal afnemen naarmate de stimulus vaker wordt gepresenteerd. Er treedt gewenning ofwel habituatie op. In een discriminatie taak worden twee of meer verschillende soorten stimuli gepresenteerd. De proefpersoon of rat moet leren verschillend te reageren op de stimuli, wat resulteert in directe stimulus-respons relaties voor elke stimulus. Het derde leer paradigma, een traditionele feature discriminatie taak, verloopt als volgt. Een zogenaamde 'target' stimulus wordt ofwel voorafgegaan door een 'feature' stimulus, ofwel alleen gepresenteerd en de proefpersoon of rat moet leren te reageren op de target als deze wordt voorafgegaan door de feature. Er wordt gezegd dat de feature 'sets the occasion', ofwel aanleiding geeft tot de aanwezigheid van een target-respons verbinding.

Het tweede gedeelte van Hoofdstuk 1 beschrijft verschillende 'event-related potentials (ERP)' van mensen en ratten. ERPs zijn elektrische hersenpotentialen die kunnen worden uitgelokt door de aanbieding van stimuli. Tevens wordt de doelmatige betekenis van deze componenten besproken. De N2 en P3 zijn componenten die veelvuldig bestudeerd zijn bij de mens. De N2 reflecteert respons inhibitie, terwijl de P3 wordt beïnvloed door factoren zoals stimulus

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waarschijnlijkheid, taak relevantie, of taak complexiteit. In ratten kan ook een P3 component worden uitgelokt, die karakteristieken laat zien lijkend op de humane P3.

Een samenvatting van de literatuur over ERP onderzoek, waarin een van de drie leer paradigma's zijn onderzocht, wordt weergegeven aan het eind van Hoofdstuk 1.

## **Habituatie**

**Hoofdstuk 2** beschrijft een experiment waarin korte en lange termijn habituatie is onderzocht bij mensen en ratten. Vier blokken van 25 auditieve stimuli werden gepresenteerd. Hoewel de latentietijden van de ERP componenten verschilden tussen de mensen en de ratten, werden twee positieve en twee negatieve ERP componenten gevonden bij beide diersoorten (P1, N1, P2, en N2). Bij zowel de mensen als de ratten, nam de amplitude van de relatief vroege componenten af binnen een blok (N1, P2, en N2 bij de mens; N1 en P2 bij de rat). Dit reflecteert wellicht korte termijn habituatie. De late ERP componenten namen af in amplitude tussen blokken (N2 bij de mensen; P2 en N2 bij de ratten) en dit reflecteert lange termijn habituatie. De resultaten wijzen erop dat er een sterke overeenkomst is in de ERP correlaten van habituatie processen van zowel mens als rat.

In het humane habituatie experiment werd verder nog een oriëntatie respons gevonden die wordt gereflecteerd in de P3 component. Deze wordt besproken in **Hoofdstuk 3**. De amplitude van deze component nam af binnen en tussen de vier blokken. Dit geeft aan dat zowel korte als lange termijn habituatie plaatsvond. Tevens was de P3 bij het begin van elk nieuw blok even groot als bij de eerste stimulus van blok 1. De amplitude van de P3 was dus hersteld. Maar tijdens de volgende stimulus presentaties nam de P3 amplitude sneller af dan in eerdere blokken. Dit effect is ook een soort habituatie en wordt 'enhanced re-habituatie' genoemd.

## **Discriminatie leren**

De volgende studies zijn uitgevoerd om de ERP componenten van mensen en ratten tijdens discriminatie taken te onderzoeken. In het experiment zoals beschreven in **Hoofdstuk 4** voerden zowel mensen als ratten een zogenoemde 'oddball' taak uit, die werd gevolgd door een passieve enkelstimulus taak.

Frequente standaard stimuli werden afgewisseld door minder frequente target stimuli in de oddball taak. De mensen werden geïnstrueerd op een knopje te drukken na het horen van de target stimuli, terwijl de ratten leerden een voerkorrel op te halen na het horen van de targets. In de passieve enkelstimulus taak werden alleen de frequente standaarden gepresenteerd, waarop de mensen en ratten niet hoefden te reageren. Zoals in het habituatie experiment werden ook nu twee positieve en twee negatieve ERP componenten gevonden bij de mensen en ratten. Tevens werd nu een derde positieve piek gevonden bij zowel de mensen, als de ratten, die groter was na de target dan na de standaard stimuli. Dit suggereert overeenkomsten in de verwerking van stimuli in discriminatie taken. Bij de mensen werd ook de N1 beïnvloed in het experiment. Deze component was negatiever na de targets dan na de standaarden in de enkelstimulus taak. Dit geeft aan dat de ERPs van mensen en ratten ook in enige mate verschillen tijdens discriminatie taken.

Een tweede oddball experiment werd uitgevoerd waarin de effecten van leren op de ERP van mensen werd getest. Deze studie wordt beschreven in **Hoofdstuk 5**. In tegenstelling tot het experiment beschreven in Hoofdstuk 4, waarin de mensen een taakinstructie kregen, moesten de mensen nu leren wanneer ze wel of niet op een stimulus dienden te reageren. De mensen leerden op een knopje te drukken na het horen van een target, maar niet naar het horen van de standaard stimulus. De N1 en P3 componenten waren in het begin van het experiment even groot tijdens de target en standaard stimuli. Op het eind, toen de mensen hadden geleerd dat ze dienden te reageren op de target, waren de N1 en P3 echter groter als reactie op target dan op standaard stimuli. De resultaten van dit experiment wijzen erop dat leren de ERP componenten van de mens beïnvloedt.

**Hoofdstuk 6** beschrijft een experiment waarin de factoren die een invloed hebben op de humane P3 component zijn onderzocht in een leerexperiment. De proefpersonen werden over drie condities verdeeld. De eerste groep leerde op een knopje te drukken na een target stimulus, maar niet na een standaard stimulus, in een oddball taak. Het interstimulus interval (ISI) in deze conditie was 9-20 s. De tweede en derde groep leerden op een knopje te drukken na alle stimuli die werden aangeboden in een enkelstimulus taak. Deze twee condities verschilden alleen met betrekking tot het ISI dat werd gebruikt; de kort-ISI groep had een ISI van 9-20 s, de lang-ISI groep kreeg een ISI van 40-90 s. Eerder onderzoek bij mensen heeft aangetoond dat het target-tot-target interval, het interval tussen twee achtereenvolgens aangeboden target stimuli, een kritieke factor is voor de P3 amplitude. In dit experiment werd echter gevonden dat het target-tot-target

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interval waarschijnlijk niet de belangrijkste factor is. Terwijl dit target-tot-target interval gelijk was in de lang-ISI en de oddball condities, lokten de stimuli in de lang-ISI conditie grotere P3 amplitudes uit dan de targets in de oddball conditie. Tevens was de P3 even groot in de kort-ISI en oddball condities, terwijl hier juist het target-tot-target interval niet even groot was. Deze resultaten geven aan dat, vooral bij lange intervallen, het interstimulus interval voor een groot deel de P3 amplitude bepaalt.

### **Feature discriminatie leren**

Een laatste serie experimenten onderzocht de ERP componenten die worden uitgelokt door een feature discriminatie taak. In een traditionele ‘serial feature-positive’ discriminatie taak wordt een target stimulus ofwel voorafgegaan door een feature, of alleen gepresenteerd. De proefpersonen of ratten moeten in deze taak leren te reageren op de target als deze wordt voorafgegaan door de feature, maar niet als deze alleen wordt aangeboden. Onderzoek heeft aangetoond dat de feature als een occasion-setter functioneert. Dat wil zeggen dat hij aanleiding geeft tot een target-respons verbinding. Feature discriminatie taken zijn nog niet vaak gebruikt bij mensen, waardoor het onduidelijk is welke cognitieve processen een rol spelen bij de mens bij dit soort taken. Daarom werden eerst twee feature discriminatie taken onderzocht bij mensen om uit te vinden welke processen nu een rol spelen. Deze experimenten worden beschreven in **Hoofdstuk 7**. In het eerste experiment leerde een groep proefpersonen te reageren op een auditieve target als deze werd voorafgegaan door een visuele feature X, maar niet als de target werd voorafgegaan door feature Y. De amplitude van de N2 en P3 componenten van de twee target stimuli verschilde niet. De Readiness Potential, een negatieve golf die cognitieve motorische voorbereiding reflecteert, liet echter wel een effect zien. De Readiness Potential was negatiever na een target waar de proefpersonen op moesten reageren dan na de targets waar ze niet op reageerden. Met betrekking tot de reacties op de feature stimulus werd het volgende gevonden. De P3 component was marginaal groter in reactie op feature X, waarop de proefpersonen indirect moesten reageren, dan op feature waarop ze niet reageerden, feature Y. Deze resultaten duiden erop dat occasion setting tegelijkertijd optrad met directe feature-respons associaties. In het tweede experiment moest een nieuwe groep proefpersonen leren op een knopje te drukken als een auditieve target A werd voorafgegaan door een visuele feature en ook als een auditieve target B alleen werd gepresenteerd. Ze leerden om niet te

reageren als target A alleen werd aangeboden of target B werd voorafgegaan door de feature. Net als in het eerste experiment werd in reactie op de target alleen een effect gevonden op de Readiness Potential. Deze was negatiever als proefpersonen reageerden op een target dan als ze niet reageerden op een target. De resultaten van deze twee experimenten suggereren dat occasion-setting processen optreden in feature discriminatie taken. Deze processen worden gereflecteerd door de Readiness Potential. De resultaten zijn tevens een aanwijzing voor het feit dat de occasion-setting processen samengaan met directe associaties.

**Hoofdstuk 8** beschrijft een studie waarin de ERPs van mensen en ratten als reactie op target stimuli in een seriële feature-positive discriminatie taak werden vergeleken. De mensen leerden op een knopje te drukken als de target werd voorafgegaan door de feature en niet op de knop te drukken als de target alleen werd gepresenteerd. De ratten leerden de voerbak te bezoeken tijdens de target stimuli die werden voorafgegaan door de features, maar niet naar de voerbak te gaan als de targets alleen werden aangeboden. Twee positieve en twee negatieve ERP componenten werden gevonden bij zowel de mensen als de ratten. De mensen vertoonden ook nog een derde positieve component. Bij zowel de mensen als de ratten was de N2 component het meest negatief als de subjecten niet hoefden te reageren, dat wil zeggen tijdens de targets die alleen werden gepresenteerd. De andere ERP componenten vertoonden geen significante taak effecten. Dit is een aanwijzing voor de overeenkomsten tussen cognitieve processen van mensen en ratten tijdens feature discriminatie taken.

De resultaten van de experimenten beschreven in dit proefschrift worden samengevat en besproken in **Hoofdstuk 9**. In het eerste gedeelte wordt de vergelijkbaarheid van de cognitieve processen tijdens de verschillende leer paradigma's behandeld. De experimenten toonden aan dat er gelijkenissen zijn in de cognitieve verwerking van stimuli, die worden gereflecteerd door de ERP componenten van mensen en ratten. De vroege ERP componenten lieten korte termijn afnamen zien na herhaalde stimulus aanbieding in het habituatie experiment en de late ERP componenten lieten vooral lange termijn afnamen zien, die habituatie reflecteerden (Hoofdstuk 2). Daarnaast was de P3 van zowel de mensen als de ratten groter na target stimuli dan na standaard stimuli in een discriminatie taak (Hoofdstuk 4). Tenslotte bleek uit de feature discriminatie taak (Hoofdstuk 8) dat de N2 component van mensen en ratten negatiever was



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als deze niet hoefden te reageren op een stimulus dan als ze wel moesten reageren.

Het tweede gedeelte van de algemene discussie bespreekt de doelmatige betekenis van de ERP componenten van mensen en ratten. In de literatuur wordt verondersteld dat de N2 component respons inhibitie reflecteert. De resultaten van de feature discriminatie taak (Hoofdstuk 8) komen overeen met dit standpunt, omdat de N2 component juist negatiever was als de mensen niet reageerden op de target stimuli dan als ze wel reageerden op de targets. De N2 component van de ratten vertoonde hetzelfde effect. Men zou daarom kunnen suggereren dat de rat N2 component dezelfde betekenis heeft.

Theoretische modellen over de humane P3 component hebben geopperd dat deze component een categorisatie van gebeurtenissen of het actualiseren van een mentaal schema reflecteert en dat de P3 component wordt beïnvloed door de waarschijnlijkheid van een stimulus, taak relevantie, en taak complexiteit. De resultaten zoals beschreven in Hoofdstuk 6 wijzen erop dat niet de waarschijnlijkheid van een stimulus de amplitude van de P3 component beïnvloedt, maar dat wellicht de tijd tussen twee stimuli in het algemeen een belangrijke factor is. De resultaten van de drie hoofdstukken over discriminatie leren (Hoofdstuk 4, 5, en 6) tonen overeenkomsten met de opinie dat taak relevantie de P3 amplitude beïnvloedt, omdat de P3 altijd groter was tijdens de target stimuli, de taak relevante stimuli dan tijdens de niet relevante standaard stimuli. Een P3 werd bij de rat alleen gevonden tijdens de discriminatie taak (Hoofdstuk 4). De suggestie wordt geopperd dat de P3 van de rat alleen de externe karakteristieken van de humane P3 reflecteert, zoals de effecten van tijd en waarschijnlijkheid, maar niet de meer geavanceerde cognitieve karakteristieken, zoals taak relevantie en complexiteit.

Samenvattend kan worden gezegd dat de N1, N2, en P3 componenten van ratten, zoals uitgelokt in leertaken, wellicht gelijke processen reflecteren als de humane N1, N2, en P3 componenten. Dit ondersteunt het standpunt dat de rat een geschikt diermodel is voor het bestuderen van de elektrofysiologische correlaten van cognitieve processen tijdens de traditionele leer paradigma's.

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- Sambeth, A., Maes, J. H. R., & Coenen, A. M. L. (submitted). A comparison of event-related potentials of humans and rats elicited by a serial feature discrimination task.

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## Curriculum Vitae

Op 16 juni 1977 werd Anke Sambeth geboren te Mönchengladbach, Duitsland. Op een leeftijd van één jaar kwam ze met haar ouders naar Nederland, waar ze eerst in Hoensbroek, en later in Amstenrade woonde. Vanaf september 1989 bezocht zij het St.-Janscollege te Hoensbroek, waar zij in juli 1995 haar VWO diploma haalde. Hierna startte ze een studie psychologie aan de Katholieke Universiteit Nijmegen. Van september 1998 tot juni 1999 liep ze stage bij de sectie Vergelijkende en Fysiologische Psychologie, met als onderwerp 'de vergelijking van de ERP van mensen en ratten tijdens discriminatieleren'. Hierna schreef ze een literatuurscriptie over het neurofysiologische correlaat van de P300 component. In december 1999 studeerde ze af in de studierichting Vergelijkende en Fysiologische Psychologie. Vervolgens werkte ze van 1 december 1999 tot 1 februari 2004 als Assistent in Opleiding bij de sectie Vergelijkende en Fysiologische Psychologie, die later werd omgedoopt tot Biologische Psychologie. Ze verrichtte onderzoek naar de vergelijkbaarheid van leerprocessen en zijn neurofysiologische correlaten tussen mensen en ratten. Tevens assisteerde ze bij onderwijs. Sinds februari 2004 is ze werkzaam bij de Cognitive Brain Research Unit te Helsinki, waar zij in samenwerking met Minna Huotilainen en Elina Pihko onderzoek verricht naar de effecten van auditieve signalen, aangeboden in discriminatietaken, op de magnetische hersenactiviteit van pasgeboren baby's.